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[Continued on next page]

(54) Title: **MODIFICATIONS OF HIV ENV, GAG, AND POL ENHANCE IMMUNOGENICITY FOR GENETIC IMMUNIZATION**

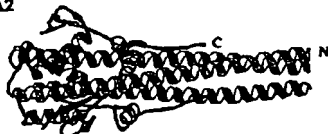
A Ebola GP2



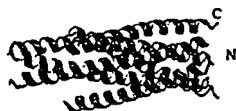
B MoMuLv Mo-55



C low-pH-treated influenza HA2



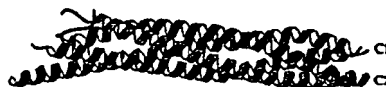
D protease resistant core of HIV gp41



E SIV gp41 (NMR)



F SNAREs



(57) Abstract: Modified HIV Env, Gag, Pol, or Nef DNA with improved ability to elicit antibody and CTL responses to HIV antigens have been identified as prototype immunogens for the treatment and prevention of HIV infections.

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Modifications of HIV Env, Gag, and Pol Enhance Immunogenicity for Genetic Immunization

5 Field of the Invention

The present invention relates to the field of molecular biology. The present invention discloses modified HIV Env, Gag, Pol, and Nef proteins, related nucleotide sequences, and usage for genetic immunization.

Background of the Invention

10 Protective immunity against human immunodeficiency virus-1 (HIV-1) is likely to require recognition of linear and conformation epitopes from multiple HIV antigens. Whether these responses can be elicited more effectively by virion-like structures or fused CTL epitopes is unknown.

The immune response to HIV infection in long-term non-progressors (Cao Y et al., 1995, *N Engl J Med*, 332:201-8; Pantaleo G et al., 1995, *N Engl J Med*, 332:209-16) and HIV-exposed sex workers suggests that specific viral immunity may limit infection and the symptoms of disease. No single characteristic yet correlates with protective immunity, but studies in non-human of primates suggest that both humoral and cellular immunity are required for this response. Depletion of cytotoxic T cells (CTLs) in chronically-infected macaques enhances viremia (Jin X et al., 1999, *J Exp Med*, 189:991-8; Schmitz JE et al., 1999, *Science*, 283:857-60). In humans, higher CTL responses correlate with lower viral load and stabilization of clinical symptoms (Musey L et al., 1997, *N Engl J Med*, 337:1267-74; Ogg GS et al., 1998, *Science*, 279:2103-6). In animal models, passive transfer of neutralizing antibodies can also contribute to protection against virus challenge (Burton DR, Montefiori DC, 1997, *AIDS*, 11 **Suppl A**:S87-S98; Gauduin MC et al., 1997, *Nat Med*, 3:1389-93; Moore JP, Ho DD., 1995, *AIDS*, 9 **Suppl A**:S117-S136; Muster T et al., 1994, *J Virol*, 68:4031-4; Muster T et al., 1993, *J Virol*, 67:6642-7; Poignard P et al., 1996, *Immunol Today*, 17:239-46; Sattentau QJ, 1996, *Curr Opin Immunol*, 8:540-5; Shibata R et al., 1999, *Nat Med*, 5:204-10). Neutralizing antibody responses can also be developed in HIV-infected individuals (Burton DR, Montefiori DC., 1997, *AIDS*, 11 **Suppl A**:S87-S98; Poignard P et al., 1996, *Immunol Today*, 17:239-46; Sattentau QJ., 1996, *Curr Opin Immunol*, 8:540-5) and are associated with lower viral loads in long-term non-progressors (Montefiori DC et al., 1996, *J Infect Dis*, 173:60-7). Though this neutralizing antibody response is

uncommon, it is directed largely against the Env protein of the virus (Robey WG et al., 1986, Proc Natl Acad Sci U S A, 83:7023-7; Steimer KS et al., 1991, Science, 254:105-8).

5 In early human vaccine trials, gp120 protein immunogens have yielded disappointing results: vaccine-induced antibodies have not been broadly neutralizing and have sometimes enhanced infection in vitro (Bolognesi DP, Matthews TJ, 1998, Nature, 391:638-9; Connor RI et al., 1998, J Virol, 72:1552-76; Haynes BF, 1996, Lancet, 348:933-7; VanCott TC et al., 1995, J Immunol, 155:4100-10). Monomeric gp120 loses oligomer-dependent epitopes and does not include sequences in the
10 ectodomain of the gp41 that become exposed during virus entry (Broder CC et al., 1994, Proc Natl Acad Sci U S A, 91:11699-703; Moore JP, 1995, Nature, 376:115; Muster T et al., 1993, J Virol, 67:6642-7). It is assumed that broadly neutralizing antibodies bind to native gp120/gp41 complex on the surface of the virus rather than soluble gp120 (Burton DR, Montefiori DC, 1997, AIDS, 11 Suppl A:S87-S98).

15 The development of a cytotoxic T lymphocyte (CTL) response to viruses is often crucial to the outcome of infections. Lysis of infected cells prior to the production of progeny virions may limit virus burst size (Yang, O et al., 1996, J. Virol., 70:5799-5806), and HIV specific CD8⁺ cytotoxic T lymphocytes (CTL) have been shown to be important in viral clearance and in the control of initial HIV-1
20 spread (Borrow, P et al., 1994, J. Virol., 68:6103-6110; Yang, O et al., 1996, J. Virol. 70:5799-5806). CTL responses specific to HIV also contribute to reduction in viral load during acute and asymptomatic infection (Klein, MR, et al., 1995, J. Exp. Med. 181:1365-1372; Moss, PAH et al., 1995, Proc. Natl. Acad. Sci. USA, 92:5773-5777) and may be involved in protection against the establishment of persistent HIV
25 infections (Rowland-Jones, SL et al., 1993, Lancet, 341:860-861; Rowland-Jones, SL et al., 1995, Nat. Med., 1:59-64). High-frequency CTL responses to HIV-1 correlated with low viral load and slow disease progression in chronically infected individuals (Musey, L et al., 1997, N. Engl. J. Med., 337:1267-1274; Ogg, GS et al., 1998, Science, 279:2103-2106.). More compelling evidence of an antiviral effect of
30 CD8⁺ cells was demonstrated in controlled studies in macaques, in which CD8⁺ cells were depleted *in vivo* using a monoclonal antibody. The viral loads in these animals increased or decreased as the CD8⁺ cells were depleted or reappeared, respectively (Jin, X et al., 1999, J. Exp. Med., 189:991-998; Schmitz, JE, et al., 1999, Science, 283:857-860). Therefore, induction of a CTL response specific to these proteins
35 represents a desirable response in an HIV-1 vaccine.

HIV-1 internal structural and enzymatic proteins contain conserved domains that preserve their functions and thus exhibit less antigenic diversity that may elicit more effective CTL responses (Nixon, DF et al., 1988, *Nature*, **336**:484-487.). Efficient and durable CTL responses require endogenous antigen synthesis and processing. Current vaccine delivery techniques include immunization with live, attenuated viruses, inactivated recombinant virus infection (Letvin, NL, 1998, *Science*, **280**:1875-1880) or plasmid DNA expression vectors. A major obstacle in the induction of CTL responses with naked DNA or recombinant virus during development of an HIV vaccine is that the expression of HIV-1 structural and enzymatic genes is tightly regulated by the virus itself. The expression of these proteins is heavily dependent upon the existence of the Rev-responsive element (RRE) of HIV-1 in recombinant vectors (Cullen, BR, 1992, *Microbiol. Rev.*, **56**:375-394; Felber, BK et al., 1989, *Proc Natl Acad Sci U S A*, **86**:1495-1499). Poor expression is caused by the presence of AT rich inhibitory nucleotide sequences (INS) in the *gag*, *pol* and *env* genes, which inhibit the nuclear export and efficient expression of unspliced HIV1 mRNAs. Early studies of DNA vaccination against HIV in mice required the inclusion of Rev in their expression vectors (Lu, S et al., 1995, *Virology*, **209**:147-154; Okuda, K et al., 1995, *AIDS Res. Hum. Retroviruses*, **11**:933-943; Wang, B et al., 1993, *Proc. Natl. Acad. Sci. U. S. A* **90**:4156-4160), but modification of INS has been shown to facilitate Rev-independent expression of HIV-1 Gag (Qiu, J-T et al., 1999, *J. Virol.*, **73**:9145-9152; zur Megede, J et al., 2000, *J Virol* **74**:2628-2635), allowing detectable humoral and CTL responses against this protein (Qiu, J-T et al., 1999, *J. Virol.*, **73**:9145-9152). These modified HIV-1 Gag genes produced viral-like particles of the expected density and morphology and induced an immune response to HIV-1 Gag after DNA immunization in mice (zur Megede, J et al., 2000, *J Virol*, **74**:2628-2635).

Summary of the Invention

Protective immunity against human immunodeficiency virus-1 (HIV-1) is likely to require recognition of linear and conformation epitopes from multiple HIV antigens, and whether these responses can be elicited more effectively by virion-like structures or fused CTL epitopes was previously unknown. Herein is provided a modified HIV Env with deletions in the cleavage site, fusogenic domain, and spacing of heptad repeats 1 and 2 to expose the core protein for optimal antigen

presentation and recognition. Additionally we provide a Gag-Pol or Gag-Pol-Nef fusion protein that is a polyprotein designed to maximize epitope presentation. The invention extends the mutation in HIV Env, Gag, Pol, and Nef to any HIV clade or strain and to related proteins of other viruses. Different combinations, different
5 orders, and different variations on a theme are envisioned, the theme being to optimize presentation of epitopes that generate broad CTL and antibody responses.

More particularly, we have investigated the effect of specific mutations in human immunodeficiency virus type 1 (HIV-1) envelope (Env) on humoral and cellular immune responses after DNA vaccination. Mice were injected with plasmid
10 expression vectors encoding HIV Env with modifications of conserved glycosylation sites or different COOH-terminal mutations intended to mimic a fusion intermediate. Elimination of conserved glycosylation sites did not substantially enhance humoral or CTL immunity. In contrast, a modified gp140 with deletions in the cleavage site, fusogenic domain and spacing of heptad repeats 1 and 2 enhanced humoral
15 immunity without reducing the efficacy of the CTL response. Because of its ability to stimulate the antibody response to native gp160 without affecting cellular immunity, this modified gp140 or a related derivative is envisioned to be a useful component of an AIDS vaccine.

In addition, we have examined the immune response to HIV-1 Gag and Pol after plasmid DNA immunization with Rev-independent expression vectors
20 encoding various forms of these proteins. Immune responses were analyzed after vaccination with four expression vectors, including Gag alone or Gag-Pol, both of which gave rise to virion-like particles (VLPs), compared to Pol alone or a Gag-Pol fusion protein that did not form VLPs. The Gag-Pol fusion protein induced the most
25 broad and potent CTL responses to Gag and Pol in DNA-vaccinated mice, and this immunogen also readily elicited an antibody response to HIV-1 Gag and Pol determinants. Through its ability to induce broad CTL and antibody responses, this Gag-Pol fusion protein or a related derivative is envisioned to be a useful component of an AIDS vaccine.

Brief Description of the Drawings

Figure A. Comparison of GP2 with the structures of viral and cellular membrane fusion proteins.

5 (A) Recombinant Ebola Zaire GP2, (B) Recombinant Mo-55 from the TM subunit of MoMuLv, (C) Low pH-treated HA2 from influenza virus, (D) Recombinant, proteolysis-resistant core of HIV-1 gp41, (E) Recombinant SIV gp41, NMR structure, (F) Recombinant core coiled segments of the SNARES syntaxin 1-A, synaptobrevin-II, and SNAP-25B. Weissenhorn et al., 1998, Molecular Cell, 2, 605-616.

10

Figure I. Schematic representation of functional domains and mutations in HIV-1 Env glycoproteins.

15 Full-length envelope polyprotein, gp160, with the indicated features based on the amino acid residues of HXB2 is shown (top). Functional domains include the gp120/gp41 cleavage site (residues 510/511), the fusion domain (512-527), the two heptad repeats (546-579 and 628-655), the transmembrane domain (684-705), and the cytoplasmic domain (706-856). The mutant forms of the envelope proteins are shown below the structure of gp160. COOH deletions were introduced that terminate the envelope protein at positions 752, 704, or 680 to produce gp150, gp145, or gp140, respectively. Two internal deletions that removed the cleavage site, the fusion domain, and the region between the two heptad repeats were introduced into gp160, gp150, gp145, and gp140. A further deletion in the COOH-terminal region at position 592 removed the second heptad repeat, the transmembrane domain, and the interspace region to produce gp128ΔCFI. To

20

25 disrupt potential glycosylation sites, asparagine (N) residues at eleven positions (88, 156, 160, 197, 230, 234, 241, 262, 276, 289, and 295) were replaced with aspartic acid (D) residues in both gp160 and gp150. Versions of both gp160 and gp150 were created with a total of 17 mutated glycosylation sites by including six additional N to D substitutions at positions 332, 339, 356, 386, 392, and 448.

30

Figure II. Comparison of the expression of the HIV-1 gp160 with codon-optimized gp160.

A. Expression of plasmids encoding Rev-dependent and Rev-independent codon-modified gp160. Upper panel: expression of Rev-dependent viral gp160 (left) and codon-modified gp160 (right) in transfected 293 cells. Lower panel: comparable expression of β -actin in these transfected cells.

B-D. Expression of mutant CXCR4- and CCR5-tropic HIV Env glycoproteins with COOH-terminal truncations (panels A and B, respectively). CXCR4-tropic envelope proteins containing mutant glycosylation sites and mutant functional domains are shown (panels C and D, respectively). The indicated proteins were detected by immunoblotting as above. Cell lysates produced by transfection with vector containing no insert were used as controls (first lane in each panel).

Figure III. Cytotoxicity of full-length gp160 is eliminated by deletion of the COOH-terminal cytoplasmic domain.

Cell rounding and detachment was not observed in control-transfected 293 cells (A), in contrast to full-length gp160 (B) and to a lesser extent in cells transfected with gp150 (C), in contrast to gp145 (D) or gp140 (E).

Figure IV. Expression of soluble gp140 Δ CFI HIV-1 envelope variant.

Immunoprecipitation and Western blot analysis of supernatants from the indicated transfected cells.

Figure V. Antibody response against HIV-1 envelope proteins in DNA immunized mice.

A. Comparison of the antibody response in mice immunized with gp140 (Δ CFI) or other Env plasmid expression vectors. Sera were collected 2 weeks after the last immunization and used to immunoprecipitate codon-altered gp160 from lysates of transfected 293 cells. The quantitation of the immunoprecipitated gp160 was done as described in Figure 5B. The average of the normalized data has been presented as a bar diagram.

B. Antibody responses in mice immunized with different mutant Env expression vectors. Antisera from immunized mice were diluted in IP buffer and 1

5 μ l of each diluted serum was used to immunoprecipitate codon-altered HIV-1 gp160 from lysates of transfected 293 cells as described in Figure 3A. The gels were scanned and the intensity of the gp160 band was determined by densitometry using the program Image Quant and presented relative to the intensity of gp160 immunoprecipitated with positive control sera (rabbit anti-gp160), which was used to normalize data between experiments. These data are presented graphically to facilitate comparison among groups.

10 C. Antibody responses in mice immunized with gp140 or gp140 (Δ CFI) were determined by immunoprecipitation and Western blotting. Animals received two booster doses (100 μ g) of the same plasmid, two weeks apart. Sera (1 μ l) collected 2 weeks after the last immunization was used to immunoprecipitate codon-optimized HIV-1 gp160 from lysates of transfected 293 cells containing 400 μ g of total protein. Each lane corresponds to the sera from an animal immunized with either the control vector (lanes 1 and 2), CXCR4-tropic gp140 (lanes 3-6), or
15 plasmid that expresses gp140 with the indicated mutant functional domains (lanes 7-10). A mouse monoclonal antibody to gp160 (HIV-1 V3 Monoclonal (IIB-V3-13), NIH AIDS Reagent Program) was used as a positive control (lane 11).

20 **Figure VI. CTL response against HIV-1 envelope proteins in DNA immunized mice.**

The CTL response to CXCR4-tropic Env and indicated deletion mutants is shown (A). The CTL responses to CXCR4-tropic envelope with glycosylation site and Δ CFI mutations are shown (B and C, respectively). Dependence of CTL activity on CD8 cells was shown by magnetic bead depletion using the indicated
25 representative immunogens (D). Spleen cells were isolated from immunized mice two weeks after the final immunization and stimulated *in vitro* with irradiated cells expressing gp160 with addition of hIL2 (5 U/ml) at day 4. The cytolytic activity of the restimulated spleen cells was tested after 7 days against V3 peptide-pulsed BC10ME cells. Similar findings were observed with target cells that stably express
30 full-length Env.

Figure VII. Schematic representation of HIV-1 Gag-Pol expression constructs.

The protein sequences of Gag (amino acids 1-432) from HXB2 (GenBank accession number K03455) and Pol (amino acids 3-1003) from NL4-3 (GenBank

accession number M19921) were used to create a synthetic version of hGag-Pol using codons found in human cells. hGag-Pol Δ FS Δ Pr was made by modification of the frame shift site (FS) and inactivation of protease. For hPol, 432 amino acids were deleted from the NH₂-terminal region of hGag-Pol and addition of an ATG codon. hGag was made by deletion of 925 amino acids from the COOH-terminal region of hGag-Pol. hGag-Pol, hGag-Pol Δ FS Δ Pr, hPol and hGag are expressed from the pNGVL-3 vector backbone.

Figure VIII. HIV-1 Gag-Pol expression in transfected 293T cells and stably transfected CT26 and BC10 ME cells.

Cell lysates from 293T cells transfected with pCMV Δ R8.2 containing viral Gag-Pol (vGag-Pol) (Naldini, L et al., 1996, Science, 272:263-267), pNGVL-hGag, hPol, hGag-Pol Δ FS Δ Pr and hGag-Pol were separated by 4-15% gradient SDS-PAGE, transferred to nitrocellulose filters, and analyzed by immunoblotting with (A) human anti HIV-1-IgG, (B) monoclonal anti-p24, and (C) rabbit anti-RT. (D) Cell lysates from CT26 and BC10ME cells stably transduced with either hGag or hPol were analyzed with human anti HIV-1-IgG.

Figure IX. Transmission electron microscopy of HIV-1 immature virus-like particles (VLP) produced by transfected 293T.

Cells were transfected with pNGVL-hGag 48 hours prior to harvesting and fixing (magnification 25,000X).

Figure X. Gag or Pol specific CTL response mediated by CD8 positive cells in immunized mice.

Two weeks after mice were immunized with a control vector, hGag, hPol, hGag-Pol Δ FS Δ Pr, and hGag-Pol, splenic cells were harvested and sensitized with naïve mouse splenic cells pulsed with Gag or Pol peptides. One week later, effector cells were tested for cytolytic activity in a 5-h ⁵¹Cr release assay using ⁵¹Cr-labeled BC10ME target cells that were pulsed for 2 hours with either (A) HIV-1 Gag peptides, or (B) HIV-1 Pol peptides. (C) CD4+ or CD8+ lymphocytes were depleted from splenic cells of immunized mice with anti-mouse- CD4+ or CD8+ Dynal beads according to the manufacturer's instructions.

Figure XI. Gag or Pol specific CTL response mediated by CD8 positive cells in immunized mice using stable expressing cell lines as target cells.

Two weeks after immunization in mice, splenic cells were harvested and sensitized with naïve mouse splenic cells pulsed with Gag or Pol peptides. One week later, effector cells were tested for cytolytic activity in a 5-h ⁵¹Cr release assay using ⁵¹Cr-labeled BC10ME target cells expressing either (A) HIV-1 Gag or (B) Pol protein.

Figure XII. HIV-1 p24 antibody ELISA assays, HIV-1 immunoblotting and immunoprecipitation Western blotting.

(A) An HIV-1 p24 antibody ELISA assay was performed by coating 96-well plates with 50 µl of purified recombinant HIV-1_{IIIIB} p24 antigen at a concentration of 2 µg/ml in PBS buffer, pH 7.4.

(B) HIV-1 immunoblotting of strips containing HIV-1 proteins were incubated with pooled mouse sera at a dilution of 1:25. Bands were visualized using the ECL western blotting detection reagent.

(C) Immunoprecipitation and Western blotting of hPol gene-transfected 293T cell lysates three days after transfection with RIPA buffer. The pooled mouse serum was diluted with IP buffer. After adding 10 µg of the cell lysate containing HIV-1 Pol protein, the reactions were incubated overnight on a rotator at 4°C. The next day, 250 µl of Protein G and A Sepharose beads (10%V/V in IP buffer) were added, and the reactions were incubated on a rotator for 2 hours at 4°C. The reactions were washed 4x with IP buffer, re-suspended with 30 µl of 1X sample buffer, and then loaded onto SDS-PAGE. The reactions were transferred to an Immobilon P membrane, and then incubated with anti HIV-1-IgG. Bands were visualized using the ECL Western blotting detection reagent.

Table 1

Plasmid	Env Plasmids		SEQ ID NO	Figure
	Plasmid Name/Description	Plasmid Map Name		
2100	pVR1012x/s R5gp139-Nef (delta) MHC (delta) CD4/h	pVR1012x/s R5gp139-Nef delta MHC delta CD4/h	1	1
2200	pVR1012x/s R5gp157-Nef (delta) MHC (delta) CD4/h	pVR1012x/s R5gp157-Nef delta MHC delta CD4/h	2	2
2300	pVR1012x/s X4 gp139-Nef delta MHC delta CD4/h	pVR1012x/s X4gp139-Nef delta MHC delta CD4/h	3	3
2302	pVR1012x/s X4gp130-Nef/h	pVR1012x/s X4gp130-Nef/h	4	4
2400	pVR1012x/s X4gp157-Nef delta MHC delta CD4/h	pVR1012x/s X4gp157-Nef delta MHC delta CD4/h	5	5
2700	pVR1012x/s X4gp140/h	pVR1012x/s X4gp140/h	6	6
2701	pVR1012x/s X4gp140 ΔCF1/h OR pVR1012x/s X4gp140(del F/CL del H IS)/h	pVR1012x/s X4gp140(del F/CL del H IS)/h	7	7
2702	pVR1012x/s X4gp128 ΔCF1/h OR pVR1012x/s X4gp128(del F/CL)/h	pVR1012x/s X4gp128(del F/CL)/h	8	8
2706	pVR1012x/s X4gp145/h	pVR1012x/s X4gp145/h	9	9
2707	pVR1012x/s X4gp145 ΔCF1/h OR pVR1012x/s X4gp145(del F/CL del H IS)/h	pVR1012x/s X4gp145(del F/CL del H IS)/h	10	10
2800	pVR1012x/s R5gp140/h	pVR1012x/s R5gp140/h	11	11
2801	pVR1012x/s R5gp140 ΔCF1/h OR pVR1012x/s R5gp140(del F/CL del H IS)/h	pVR1012x/s R5gp140(del F/CL del H IS)/h	12	12
2804	pVR1012x/s R5gp145/h	pVR1012x/s R5gp145/h	13	13
2805	pVR1012x/s R5gp145 ΔCF1/h OR pVR1012x/s R5gp145(del F/CL del H IS)/h	pVR1012x/s R5gp145(del F/CL del H IS)/h	14	14
2810	pVR1012x/s R5gp140delC1(delCFI)/h	pVR1012x/s R5gp140delC1(delCFI)/h	15	15
2811	pVR1012x/s R5gp140delC2(delCFI)/h	pVR1012x/s R5gp140delC2(delCFI)/h	16	16
2812	pVR1012x/s R5gp140delC3(delCFI)/h	pVR1012x/s R5gp140delC3(delCFI)/h	17	17
2813	pVR1012x/s R5gp140delC4 (delCFI)/h	pVR1012x/s R5gp140delC4 (delCFI)/h	18	18
2814	pVR1012x/s R5gp140delC5 (delCFI)/h	pVR1012x/s R5gp140delC5 (delCFI)/h	19	19
2820	pVR1012x/s R5gp140(dCFI)/dv1	pVR1012x/s R5gp140(dCFI)/dv1/h	20	20
2821	pVR1012x/s R5gp140(dCFI)/dv2	pVR1012x/s R5gp140(dCFI)/dv2/h	21	21
2822	pVR1012x/s R5gp140(dCFI)/dv3	pVR1012x/s R5gp140(dCFI)/dv3/h	22	22
2823	pVR1012x/s R5gp140(dCFI)/dv4	pVR1012x/s R5gp140(dCFI)/dv4/h	23	23
2824	pVR1012x/s R5gp140(dCFI)/dv12	pVR1012x/s R5gp140(dCFI)/dv12/h	24	24
2825	pVR1012x/s R5gp140(dCFI)/dv13	pVR1012x/s R5gp140(dCFI)/dv13/h	25	25
2826	pVR1012x/s R5gp140(dCFI)/dv14	pVR1012x/s R5gp140(dCFI)/dv14/h	26	26
2827	pVR1012x/s R5gp140(dCFI)/dv23	pVR1012x/s R5gp140(dCFI)/dv23/h	27	27
2828	pVR1012x/s R5gp140(dCFI)/dv24	pVR1012x/s R5gp140(dCFI)/dv24/h	28	28
2829	pVR1012x/s R5gp140(dCFI)/dv34	pVR1012x/s R5gp140(dCFI)/dv34/h	29	29
2830	pVR1012x/s R5gp140(dCFI)/dv123	pVR1012x/s R5gp140(dCFI)/dv123/h	30	30

Plasmid	Env Plasmids		Plasmid Map Name	SEQ ID NO	Figure
	Plasmid Name/Description				
2831	pVR1012x/s R5gp140(dCFI)/dv124		pVR1012x/s R5gp140(dCFI)/dv124/h	31	31
2832	pVR1012x/s R5gp140(dCFI)/dv134		pVR1012x/s R5gp140(dCFI)/dv134/h	32	32
2833	pVR1012x/s R5gp140(dCFI)/dv234		pVR1012x/s R5gp140(dCFI)/dv234/h	33	33
2834	pVR1012x/s R5gp140(dCFI)/dv1234		pVR1012x/s R5gp140(dCFI)/dv1234/h	34	34
2835	pAdApt R5gp140(dCFI)/dv1		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv1/h	35	35
2836	pAdApt R5gp140(dCFI)/dv2		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv2/h	36	36
2837	pAdApt R5gp140(dCFI)/dv3		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv3/h	37	37
2838	pAdApt R5gp140(dCFI)/dv4		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv4/h	38	38
2839	pAdApt R5gp140(dCFI)/dv12		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv12/h	39	39
2840	pAdApt R5gp140(dCFI)/dv13		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv13/h	40	40
2841	pAdApt R5gp140(dCFI)/dv14		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv14/h	41	41
2842	pAdApt R5gp140(dCFI)/dv23		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv23/h	42	42
2843	pAdApt R5gp140(dCFI)/dv24		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv24/h	43	43
2844	pAdApt R5gp140(dCFI)/dv34		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv34/h	44	44
2845	pAdApt R5gp140(dCFI)/dv123		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv123/h	45	45
2846	pAdApt R5gp140(dCFI)/dv124		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv124/h	46	46
2847	pAdApt R5gp140(dCFI)/dv134		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv134/h	47	47
2848	pAdApt R5gp140(dCFI)/dv234		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv234/h	48	48
2849	pAdApt R5gp140(dCFI)/dv1234		pAdApt CMV TbGH (+) R5gp140(dCFI)/dv1234/h	49	49
2850	pVR1012x/s R5gp145delC1 (delCFI)/h		pVR1012x/s R5gp145delC1 (delCFI)/h	50	50
2851	pVR1012x/s R5gp145delC2 (delCFI)/h		pVR1012x/s R5gp145delC2 (delCFI)/h	51	51
2852	pVR1012x/s R5gp145delC3 (delCFI)/h		pVR1012x/s R5gp145delC3 (delCFI)/h	52	52
2853	pVR1012x/s R5gp145delC4 (delCFI)/h		pVR1012x/s R5gp145delC4 (delCFI)/h	53	53
2854	pVR1012x/s R5gp145delC5 (delCFI)/h		pVR1012x/s R5gp145delC5 (delCFI)/h	54	54
2860	pVR1012x/s R5gp145(dCFI)/h/dV1		pVR1012x/s R5 gp145(dCFI)/dv1/h	55	55
2861	pVR1012x/s R5gp145(dCFI)/h/dV2		pVR1012x/s R5gp145(dCFI)/dv2/h	56	56
2862	pVR1012x/s R5gp145(dCFI)/h/dV3		pVR1012x/s R5gp145(dCFI)/dv3/h	57	57
2863	pVR1012x/s R5gp145(dCFI)/h/dV4		pVR1012x/s R5gp145(dCFI)/dv4/h	58	58
2864	pVR1012x/s R5gp145(dCFI)/h/dV12		pVR1012x/s R5gp145(dCFI)/dv12/h	59	59
2865	pVR1012x/s R5gp145(dCFI)/h/dV13		pVR1012x/s R5gp145(dCFI)/dv13/h	60	60
2866	pVR1012x/s R5gp145(dCFI)/h/dV14		pVR1012x/s R5gp145(dCFI)/dv14/h	61	61
2867	pVR1012x/s R5gp145(dCFI)/h/dV23		pVR1012x/s R5gp145(dCFI)/dv23/h	62	62

Env Plasmids			Plasmid Map Name	SEQ ID NO	Figure
Plasmid	Plasmid Name/Description				
2868	pVR1012x/s R5gp145(dCFI)/h/dV24		pVR1012x/s R5gp145(dCFI)/dv24/h	63	63
2869	pVR1012x/s R5gp145(dCFI)/h/dV34		pVR1012x/s R5gp145(dCFI)/dv34/h	64	64
2870	pVR1012x/s R5gp145(dCFI)/h/dV134		pVR1012x/s R5gp145(dCFI)/dv134/h	65	65
2871	pVR1012x/s R5gp145(dCFI)/h/dV234		pVR1012x/s R5gp145(dCFI)/dv234/h	66	66
2872	pVR1012x/s R5gp145(dCFI)/h/dV123/h		pVR1012x/s R5gp145(dCFI)/dv123/h	67	67
2873	pVR1012x/s R5gp145(dCFI)/h/dV124		pVR1012x/s R5gp145(dCFI)/dv124/h	68	68
2874	pVR1012x/s R5gp145(dCFI)/h/dV1234		pVR1012x/s R5gp145(dCFI)/dv1234/h	69	69
2900	pVR1012x/s R5gp150/h		pVR1012x/s R5gp150/h	70	70
3000	pVR1012x/s R5gp160/h		pVR1012x/s R5gp160/h	71	71
3200	pVR1012x/s X4gp150/h		pVR1012x/s X4gp150/h	72	72
3201	pVR1012x/s X4gp150 ΔCFI/h OR pVR1012x/s X4gp150(dcl F/CL del H IS)/h		pVR1012x/s X4gp150(dcl F/CL del H IS)/h	73	73
3202	pVR1012x/s X4gp150 Δgly/h		pVR1012x/s X4gp150 delta gly	74	74
3203	pVR1012x/s X4gp150 AB Δgly/h		pVR1012x/s X4gp150 AB (delta)gly/h	75	75
3300	pVR1012x/s X4gp160/h		pVR1012x/s X4gp160/h	76	76
3301	pVR1012x/s X4gp160 ΔCFI/h OR pVR1012x/s X4gp160(dcl F/CL del H IS)/h		pVR1012x/s X4gp160(dcl F/CL del H IS)/h	77	77
3400	pVR1012x/s X4gp160 Δgly/h		pVR1012x/s X4gp160 delta gly	78	78
3401	pVR1012x/s X4gp160 AB Δgly/h OR pVR1012x/s X4gp160AB mut Δgly/h		pVR1012x/s X4gp160 AB Dgly/h	79	79
3500	pVR1012x/s Nef/h		pVR1012x/s Nef/h	80	80
3600	pVR1012x/s NefDMHCD4/h		pVR1012x/s Nef delta MHC delta CD4/h	81	81
3700	pVR1012x/s NefDCD4/h		pVR1012x/s Nef delta CD4/h	82	82
3800	pVR1012x/s NefDMHC/h		pVR1012x/s Nef delta MHC/h	83	83
5200	pVR1012 x/s 89.6Pgp128(dcl F/CL)/h		pVR1012 x/s 89.6Pgp128(dcl F/CL)/h	84	84
5201	R5 Clade 89.6P gp140 ΔCFI/h		pVR1012x/s 89.6Pgp140(dcl F/CL del H IS)/h	85	85
5202	pVR1012 x/s 89.6Pgp145(dcl F/CL del H IS)/h		pVR1012 x/s 89.6Pgp145(dcl F/CL del H IS)/h	86	86
5203	pVR1012 x/s 89.6Pgp160/h		pVR1012 x/s 89.6Pgp160/h	87	87
5300	R5 Clade C gp140 ΔCFI/h OR pVR1012x/s R5 (cladeC)gp140(dcl F/CL del H IS)/h		pVR1012x/s CladeC(R5)gp140(dcl F/CL del H IS)/h	88	88
5301	pVR1012x/s R5(cladeC)gp145(dcl F/CL del H IS)/h		pVR1012x/s CladeC(R5)gp145(dcl F/CL del H IS)/h	89	89
5303	pVR1012x/s R5gp145 CladeC (Brazil) delCFI/h		pVR1012x/s R5gp145 CladeC (Brazil) delCFI/h	90	90
5304	pVR1012x/s R5(clade A)gp140(dcl F/CL del H IS)/h		pVR1012x/s R5gp140CladeA (dCFI)/h	91	91
5305	pVR1012x/s R5(clade A)gp145(dcl F/CL del H IS)/h		pVR1012x/s R5gp145CladeA (dCFI)/h	92	92
5306	pVR1012x/s R5(clade E)gp140(dcl F/CL del H IS)/h		pVR1012x/s R5gp140 Clade E (dCFI)/h	93	93
5307	pVR1012x/s R5(clade E)gp145(dcl F/CL del H IS)/h		pVR1012x/s R5 gp145 Clade E (dCFI)/h	94	94

Plasmid	Plasmid Name/Description	Env Plasmids	Plasmid Map Name	SEQ ID NO	Figure
5308	pVR1012x/s R5/clade C South African)gp140(del F/CL del H IS)/h		pVR1012x/s R5gp140 Clade C(SA) (dCFI)/h	95	95
5309	pVR1012x/s R5/clade C South African)gp145(del F/CL del H IS)/h		pVR1012x/s R5gp145 CladeC(SA) (dCFI)/h	96	96
5350	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv1		pVR1012x/s R5gp140CladeC (Brazil) dCFIdv1/h	97	97
5351	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv12		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv12/h	98	98
5352	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv123		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv123/h	99	99
5353	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv1234		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv1234/h	100	100
5354	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv124		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv124/h	101	101
5355	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv13		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv13/h	102	102
5356	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv134		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv134/h	103	103
5357	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv14		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv14/h	104	104
5358	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv2		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv2/h	105	105
5359	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv23		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv23/h	106	106
5360	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv234		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv234/h	107	107
5361	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv24		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv24/h	108	108
5362	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv3		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv3/h	109	109
5363	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv34		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv34/h	110	110
5364	pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dv4		pVR1012x/s R5gp140CladeC (Brazil) (dCFI)dv4/h	111	111
5365	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv1		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv1/h	112	112
5366	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv12		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv12/h	113	113
5367	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv123		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv123/h	114	114
5368	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv1234		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv1234/h	115	115
5369	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv124		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv124/h	116	116
5370	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv13		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv13/h	117	117
5371	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv134		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv134/h	118	118
5372	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv14		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv14/h	119	119
5373	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv2		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv2/h	120	120
5374	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv23		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv23/h	121	121
5375	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv234		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv234/h	122	122
5376	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv24		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv24/h	123	123
5377	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv3		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv3/h	124	124
5378	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv34		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv34/h	125	125
5379	pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dv4		pVR1012x/s R5gp145CladeC (Brazil) (dCFI)dv4/h	126	126

Env Plasmids			Plasmid Map Name	SEQ ID NO	Figure
Plasmid	Plasmid Name/Description				
5500	pVR1012x/s R5(SA-C) gp140(dCFI) dV1/h		pVR1012x/s R5 gp140(dCFI) SA dV1/h	127	127
5501	pVR1012x/s R5(SA-C) gp140(dCFI) dV12/h		pVR1012x/s R5 gp140(dCFI) SA dV12/h	128	128
5502	pVR1012x/s R5(SA-C) gp140(dCFI) dV123/h		pVR1012x/s R5 gp140(dCFI) SA dV123/h	129	129
5503	pVR1012x/s R5(SA-C) gp140(dCFI) dV1234/h		pVR1012x/s R5 gp140(dCFI) SA dV1234/h	130	130
5504	pVR1012x/s R5(SA-C) gp140(dCFI) dV124/h		pVR1012x/s R5 gp140(dCFI) SA dV124/h	131	131
5505	pVR1012x/s R5(SA-C) gp140(dCFI) dV13/h		pVR1012x/s R5 gp140(dCFI) SA dV13/h	132	132
5506	pVR1012x/s R5(SA-C) gp140(dCFI) dV134/h		pVR1012x/s R5 gp140(dCFI) SA dV134/h	133	133
5507	pVR1012x/s R5(SA-C) gp140(dCFI) dV14/h		pVR1012x/s R5 gp140(dCFI) SA dV14/h	134	134
5508	pVR1012x/s R5(SA-C) gp140(dCFI) dV2/h		pVR1012x/s R5 gp140(dCFI) SA dV2/h	135	135
5509	pVR1012x/s R5(SA-C) gp140(dCFI) dV23/h		pVR1012x/s R5 gp140(dCFI) SA dV23/h	136	136
5510	pVR1012x/s R5(SA-C) gp140(dCFI) dV234/h		pVR1012x/s R5 gp140(dCFI) SA dV234/h	137	137
5511	pVR1012x/s R5(SA-C) gp140(dCFI) dV24/h		pVR1012x/s R5 gp140(dCFI) SA dV24/h	138	138
5512	pVR1012x/s R5(SA-C) gp140(dCFI) dV3/h		pVR1012x/s R5 gp140(dCFI) SA dV3/h	139	139
5513	pVR1012x/s R5(SA-C) gp140(dCFI) dV34/h		pVR1012x/s R5 gp140(dCFI) SA dV34/h	140	140
5514	pVR1012x/s R5(SA-C) gp140(dCFI) dV4/h		pVR1012x/s R5 gp140(dCFI) SA dV4/h	141	141
5515	pVR1012x/s R5(SA-C) gp145(dCFI) dV1/h		pVR1012x/s R5 gp145(dCFI) SA dV1/h	142	142
5516	pVR1012x/s R5(SA-C) gp145(dCFI) dV12/h		pVR1012x/s R5 gp145(dCFI) SA dV12/h	143	143
5517	pVR1012x/s R5(SA-C) gp145(dCFI) dV123/h		pVR1012x/s R5 gp145(dCFI) SA dV123/h	144	144
5518	pVR1012x/s R5(SA-C) gp145(dCFI) dV1234/h		pVR1012x/s R5 gp145(dCFI) SA dV1234/h	145	145
5519	pVR1012x/s R5(SA-C) gp145(dCFI) dV2/h		pVR1012x/s R5 gp145(dCFI) SA dV2/h	146	146
5520	pVR1012x/s R5(SA-C) gp145(dCFI) dV23/h		pVR1012x/s R5 gp145(dCFI) SA dV23/h	147	147
5521	pVR1012x/s R5(SA-C) gp145(dCFI) dV234/h		pVR1012x/s R5 gp145(dCFI) SA dV234/h	148	148
5522	pVR1012x/s R5(SA-C) gp145(dCFI) dV24/h		pVR1012x/s R5 gp145(dCFI) SA dV24/h	149	149
5523	pVR1012x/s R5(SA-C) gp145(dCFI) dV3/h		pVR1012x/s R5 gp145(dCFI) SA dV3/h	150	150
5524	pVR1012x/s R5(SA-C) gp145(dCFI) dV34/h		pVR1012x/s R5 gp145(dCFI) SA dV34/h	151	151
5525	pVR1012x/s R5(SA-C) gp145(dCFI) dV4/h		pVR1012x/s R5 gp145(dCFI) SA dV4/h	152	152
5526	pVR1012x/s R5(SA-C) gp145(dCFI) dV13/h		pVR1012x/s R5 gp145(dCFI) SA dV13/h	153	153
5527	pVR1012x/s R5(SA-C) gp145(dCFI) dV134/h		pVR1012x/s R5 gp145(dCFI) SA dV134/h	154	154
5528	pVR1012x/s R5(SA-C) gp145(dCFI) dV124/h		pVR1012x/s R5 gp145(dCFI) SA dV124/h	155	155
5529	pVR1012x/s R5(SA-C) gp145(dCFI) dV14/h		pVR1012x/s R5 gp145(dCFI) SA dV14/h	156	156

Gag/Pol Plasmids			Plasmid Map Name	SEQ ID NO	Figure
Plasmid	Plasmid Name/Description				
3900	pVR1012x/s HIV Gag /h		pVR1012x/s Gag/h	157	157
3901	pVR1012x/s SIV Gag /h		pVR1012x/s SIV Gag/h	158	158
4000	pVR1012x/s HIV Gag-Pol ΔFS ΔPR /h OR pVR1012x/s Gag-Pol/h		pVR1012x/s Gag-PolΔFSΔPR/h	159	159
4001	pVR1012x/s SIV Gag-Pol /h		pVR1012x/s SIV Gag-Pol/h	160	160
4100	pVR1012x/s HIV Pol /h		pVR1012x/s Pol/h	161	161
4101	pVR1012x/s SIV Pol /h		pVR1012x/s SIV Pol/h	162	162
4200	pVR1012x/s HIV Gag-Pol /h		pVR1012x/s Gag(s)Pol/h	163	163
4300	pVR1012x/s HIV Gag-Pol ΔRT ΔIN /h OR pVR1012 Gag-Pol(d delta RT delta IN)/h		pVR1012x/s Gag-Pol(fS)RT(-)IN(-)	164	164
4301	pVR1012x/s-Cag(FS)-Pol-delta RT IN-RES R5 gp157-Nef		pVR1012x/s-Gag(FS)-Pol-delta RT IN-RES-R5 gp157-Nef	165	165
4302	pVR1012x/s HIV gag-Pol ΔFS ΔPR ΔRT ΔIN /h		pVR1012x/s Gag(dclFS)Pol (delta PR delta RT delta IN)/h	166	166
4303	pVR1012x/s SIV Gag-Pol ΔFS /h OR pVR1012 SIV Gag(dclFS)Pol(delta PR delta RT delta IN)/h		pVR1012x/s SIV Gag(dclFS)-Pol/h	167	167
4304	pVR1012 Gag (dclFS)Pol delta PR delta RT delta IN/h		pVR1012 Gag-C(dclFS)Pol (deltaPR deltaRT deltaIN)/h	168	168
4305	pVR1012 Gag-A(dclFS)Pol(delta PR delta RT delta IN)/h		pVR1012 Gag-A(dclFS)Pol (deltaPR delta RT deltaIN)/h	169	169
4306	pVR1012 Gag(dclFS)Pol delta PR delta RT delta IN/Nef/h		pVR1012 Gag(dclFS)Pol deltaPR deltaRT deltaIN/Nef/h	170	170
4308	pVR1012 Gag (dclFS) Pol deltaPR deltaRT deltaIN deltaMyr/h		pVR1012 Gag(dclFS)Pol deltaPR deltaRT deltaIN delta Myr /h	171	171
4309	pVR1012 Gag (dclFS) Pol deltaPR deltaRT deltaIN delta Myr/Nef/h		pVR1012x/s Gag(dclFS)Pol deltaPR deltaRT deltaIN delta Myr/Nef/h	172	172
4310	pVR1012 Nef Gag (del fs) (del Myr) Pol (delta PR delta RT delta IN)/h		pVR1012 Nef Gag(dclFS)(del Myr) Pol (delta PR delta RT delta IN)/h	173	173
4311	pVR1012 Gag-C(dclFS)Pol(delta PR delta RT delta IN) Nef/h		pVR1012 Gag-C(dclFS) Pol(delta PR delta RT delta IN) Nef/h	174	174
4312	Gag (del fs) (del Myr) Nef Pol ΔPRΔRTΔIN/h		pVR1012 Gag (del FS) (del Myr) Nef Pol (delta PR delta RT delta IN)/h	175	175
4313	pVR1012 Gag Clade A (del fs)Pol(Δ PR Δ RT Δ IN)/h		pVR1012 Gag-A (del FS) Pol (delPR delRT delIN) Nef/h	176	176

Detailed Description of the Invention

The present invention provides modifications of HIV Env, Gag, Pol, and Nef that enhance immunogenicity for genetic immunization. Both HIV and SIV are genetically related members of the lentivirus genus of the *Retroviridae* family. Lentivirus isolates from humans are grouped into one of two types, designated HIV-1 and HIV-2. A classification scheme recognizes nine subtypes (clades) of HIV-1 (A through I) and five subtypes of HIV-2 (A through E). A compendium of HIV and SIV sequence information is found in a database prepared by Myers et al., Los Alamos, NM: Los Alamos National Laboratory.

10 **Nucleic Acid Molecules**

As indicated herein, nucleic acid molecules of the present invention may be in the form of RNA or in the form of DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

25 Nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) of a wild-type HIV gene; and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode an ORF of a wild-type HIV polypeptide. Of course, the genetic code is well known in the art. Degenerate variants optimized for human codon usage are preferred.

30 In another aspect, the invention provides a nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above. By "stringent hybridization conditions" is intended overnight incubation at 42 degree C in a solution comprising: 50% formamide, 5 times SSC (750 mM NaCl, 75

mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 times Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1 times SSC at about 65 degree C.

5 By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide.

10 By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide. Of course, a polynucleotide which hybridizes only to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule
15 containing a poly T (or U) stretch or the complement thereof (e.g., practically any double-stranded DNA clone).

As indicated herein, nucleic acid molecules of the present invention which encode an HIV polypeptide may include, but are not limited to those encoding the amino acid sequence of the full-length polypeptide, by itself, the coding sequence
20 for the full-length polypeptide and additional sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro-protein sequence, the coding sequence of the full-length polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences,
25 such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example, ribosome binding and stability of mRNA; and additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities.

30 The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the HIV protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a genome of an organism. Genes II, Lewin, B., ed., John Wiley & Sons, New

York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions, which may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the HIV polypeptide or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to a nucleotide sequence encoding a polypeptide having the amino acid sequence of a wild-type HIV polypeptide or a nucleotide sequence complementary thereto.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a HIV polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the HIV polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to the reference nucleotide sequence can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). Bestfit uses the local homology algorithm of Smith and Waterman,

Advances in Applied Mathematics 2: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters
5 are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences shown herein in the
10 Sequence Listing which encode a polypeptide having HIV polypeptide activity. By "a polypeptide having HIV activity" is intended polypeptides exhibiting HIV activity in a particular biological assay. For example, Env, Gag, and Pol protein activity can be measured for changes in immunological character by an appropriate immunological assay.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in
15 the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 95%, 96%, 97%, 98%, or 99% identical to a nucleic acid sequence shown herein in the Sequence Listing will encode a polypeptide "having HIV polypeptide activity." In fact, since degenerate variants of these nucleotide
20 sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having HIV polypeptide activity. This is because the skilled artisan is fully aware of amino acid
25 substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., "Deciphering the Message in
30 Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Polypeptides and Fragments

The invention further provides a HIV polypeptide having the amino acid sequence encoded by an open reading frame (ORF) of a wild-type HIV gene, or a peptide or polypeptide comprising a portion thereof (e.g., gp120).

5 It will be recognized in the art that some amino acid sequences of the HIV polypeptides can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity.

10 Thus, the invention further includes variations of the HIV polypeptide which show substantial HIV polypeptide activity or which include regions of HIV protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J. U., et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990).

15 Thus, the fragment, derivative or analog of the polypeptide of the invention may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are
20 deemed to be within the scope of those skilled in the art from the teachings herein.

25 As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table A).

Table A
Conservative Amino Acid Substitutions

Aromatic	Phenylalanine Tryptophan Tyrosine
Ionizable: Acidic	Aspartic Acid Glutamic Acid
Ionizable: Basic	Arginine Histidine Lysine
Nonionizable Polar	Asparagine Glutamine Selenocystine Serine Threonine
Nonpolar (Hydrophobic)	Alanine Glycine Isoleucine Leucine Proline Valine
Sulfur Containing	Cysteine Methionine

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions for any given HIV polypeptide will not be more than 50, 40, 30, 20, 10, 5 or 3.

Amino acids in the HIV polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as changes in immunological character.

The polypeptides of the present invention are conveniently provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention.

5 Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell or a native source. For example, a recombinantly produced version of the HIV polypeptide can be substantially purified by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988).

10 The polypeptides of the present invention include a polypeptide comprising a polypeptide shown herein in the Sequence Listing; as well as polypeptides which are at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to those described above and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

15 By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of an HIV polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the HIV polypeptide. In other words, to obtain a polypeptide having an amino acid sequence
20 at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These
25 alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 95%,
30 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown herein in the Sequence Listing can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). When using Bestfit or any other
35 sequence alignment program to determine whether a particular sequence is, for

instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The polypeptides of the invention may be produced by any conventional means. Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl. Acad. Sci. USA 82:5131-5135. This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Pat. No. 4,631,211 to Houghten et al. (1986).

The present invention also relates to vectors which include the nucleic acid molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of HIV polypeptides or fragments thereof by recombinant techniques.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells; insect cells such

as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986).

The HIV polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Pharmaceutical Formulations, Dosages, and Modes of Administration

The compounds of the invention may be administered using techniques well known to those in the art. Preferably, compounds are formulated and administered by genetic immunization. Techniques for formulation and administration may be found in "Remington's Pharmaceutical Sciences", 18th ed., 1990, Mack Publishing Co., Easton, PA. Suitable routes may include parenteral delivery, such as

intramuscular, intradermal, subcutaneous, intramedullary injections, as well as, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few. For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer.

In instances wherein intracellular administration of the compounds of the invention is preferred, techniques well known to those of ordinary skill in the art may be utilized. For example, such compounds may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are effectively delivered into the cell cytoplasm.

Nucleotide sequences of the invention which are to be intracellularly administered may be expressed in cells of interest, using techniques well known to those of skill in the art. For example, expression vectors derived from viruses such as retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, vaccinia viruses, polio viruses, or sindbis or other RNA viruses, or from plasmids may be used for delivery and expression of such nucleotide sequences into the targeted cell population. Methods for the construction of such expression vectors are well known. See, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY, and Ausubel et al., 1989, Current Protocols In Molecular Biology, Greene Publishing Associates and Wiley Interscience, NY.

The invention extends to the use of a plasmid for primary immunization (priming) of a host and the subsequent use of a recombinant virus, such as a retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, polio virus, or sindbis or other RNA virus, for boosting said host, and vice versa. For example, the host may be immunized (primed) with a plasmid by DNA immunization and receive a boost with the corresponding viral construct, and vice versa. Alternatively, the host may be immunized (primed) with a plasmid by DNA immunization and receive a boost with not the corresponding viral construct but a different viral construct, and vice versa.

With respect to HIV Env, Gag, and Pol, protein sequences of the invention may be used as therapeutics or prophylactics (as subunit vaccines) in the treatment of AIDS or HIV infection. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that includes the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (e.g., the concentration of the test compound which achieves a half-maximal inhibition of viral infection relative to the amount of the event in the absence of the test compound) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography (HPLC).

The compounds of the invention may, further, serve the role of a prophylactic vaccine, wherein the host produces antibodies and/or CTL responses against HIV Env, Gag and Pol, which responses then preferably serve to neutralize HIV viruses by, for example, inhibiting further HIV infection. Administration of the compounds of the invention as a prophylactic vaccine, therefore, would comprise administering to a host a concentration of compounds effective in raising an immune response which is sufficient to elicit antibody and/or CTL responses to HIV Env, Gag, and Pol, and/or neutralize HIV, by, for example, inhibiting HIV ability to infect cells. The exact concentration will depend upon the specific compound to be administered, but may be determined by using standard techniques for assaying the development of an immune response which are well known to those of ordinary skill in the art.

The compounds may be formulated with a suitable adjuvant in order to enhance the immunological response. Such adjuvants may include, but are not limited to mineral gels such as aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols, polyanions; other peptides; oil emulsions; and
5 potentially useful human adjuvants such as BCG and *Corynebacterium parvum*.

Adjuvants suitable for co-administration in accordance with the present invention should be ones that are potentially safe, well tolerated and effective in people including QS-21, Detox-PC, MPL-SE, MoGM-CSF, TiterMax-G, CRL-1005, GERBU, TERamide, PSC97B, Adjuver, PG-026, GSK-1, GcMAF, B-
10 alethine, MPC-026, Adjuvax, CpG ODN, Betafectin, Alum, and MF59 (see Kim et al., 2000, Vaccine, 18: 597 and references therein).

Other contemplated adjuvants that may be administered include lectins, growth factors, cytokines and lymphokines such as alpha-interferon, gamma-interferon, platelet derived growth factor (PDGF), gCSF, gMCSF, TNF, epidermal
15 growth factor (EGF), IL-1, IL-2, IL-4, IL-6, IL-8, IL-10 and IL-12.

For all such treatments described above, the exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p. 1).

20 It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the viral
25 infection of interest will vary with the severity of the condition to be treated and the route of administration. The dose and perhaps prime-boost regimen, will also vary according to the age, weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

The pharmacologically active compounds of this invention can be processed
30 in accordance with conventional methods of galenic pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans.

The compounds of this invention can be employed in admixture with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral (e.g., oral) or topical application
35 which do not deleteriously react with the active compounds. Suitable

pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatine, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds. They can also be combined where desired with other active agents, e.g., vitamins.

For parenteral application, which includes intramuscular, intradermal, subcutaneous, intranasal, intracapsular, intraspinal, intrasternal, and intravenous injection, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

For enteral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules. The pharmaceutical compositions may be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated

edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. A syrup, elixir, or the like can be used wherein a sweetened vehicle is employed.

Sustained or directed release compositions can be formulated, e.g., liposomes or those wherein the active compound is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc. It is also possible to freeze dry the new compounds and use the lyophilizates obtained, for example, for the preparation of products for injection.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

For topical application, there are employed as non-sprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, aerosols, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g., a freon.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

Genetic Immunization

Genetic immunization according to the present invention elicits an effective immune response without the use of infective agents or infective vectors. Vaccination techniques which usually do produce a CTL response do so through the use of an infective agent. A complete, broad based immune response is not generally exhibited in individuals immunized with killed, inactivated or subunit vaccines. The present invention achieves the full complement of immune responses in a safe manner without the risks and problems associated with vaccinations that use infectious agents.

According to the present invention, DNA or RNA that encodes a target protein is introduced into the cells of an individual where it is expressed, thus producing the target protein. The DNA or RNA is linked to regulatory elements necessary for expression in the cells of the individual. Regulatory elements for DNA include a promoter and a polyadenylation signal. In addition, other elements, such as a Kozak region, may also be included in the genetic construct.

The genetic constructs of genetic vaccines comprise a nucleotide sequence that encodes a target protein operably linked to regulatory elements needed for gene expression. Accordingly, incorporation of the DNA or RNA molecule into a living cell results in the expression of the DNA or RNA encoding the target protein and thus, production of the target protein.

When taken up by a cell, the genetic construct which includes the nucleotide sequence encoding the target protein operably linked to the regulatory elements may remain present in the cell as a functioning extrachromosomal molecule or it may integrate into the cell's chromosomal DNA. DNA may be introduced into cells where it remains as separate genetic material in the form of a plasmid. Alternatively, linear DNA which can integrate into the chromosome may be introduced into the cell. When introducing DNA into the cell, reagents which promote DNA integration into chromosomes may be added. DNA sequences which are useful to promote integration may also be included in the DNA molecule. Since integration into the chromosomal DNA necessarily requires manipulation of the chromosome, it is preferred to maintain the DNA construct as a replicating or non-replicating extrachromosomal molecule. This reduces the risk of damaging the cell by splicing into the chromosome without affecting the effectiveness of the vaccine. Alternatively, RNA may be administered to the cell. It is also contemplated to

provide the genetic construct as a linear minichromosome including a centromere, telomeres and an origin of replication.

5 The necessary elements of a genetic construct of a genetic vaccine include a nucleotide sequence that encodes a target protein and the regulatory elements necessary for expression of that sequence in the cells of the vaccinated individual. The regulatory elements are operably linked to the DNA sequence that encodes the target protein to enable expression.

10 The molecule that encodes a target protein is a protein-encoding molecule which is translated into protein. Such molecules include DNA or RNA which comprise a nucleotide sequence that encodes the target protein. These molecules may be cDNA, genomic DNA, synthesized DNA or a hybrid thereof or an RNA molecule such as mRNA. Accordingly, as used herein, the terms "DNA construct", "genetic construct" and "nucleotide sequence" are meant to refer to both DNA and RNA molecules.

15 The regulatory elements necessary for gene expression of a DNA molecule include: a promoter, an initiation codon, a stop codon, and a polyadenylation signal. In addition, enhancers are often required for gene expression. It is necessary that these elements be operable in the vaccinated individual. Moreover, it is necessary that these elements be operably linked to the nucleotide sequence that encodes the target protein such that the nucleotide sequence can be expressed in the cells of a vaccinated individual and thus the target protein can be produced.

Initiation codons and stop codons are generally considered to be part of a nucleotide sequence that encodes the target protein. However, it is necessary that these elements are functional in the vaccinated individual.

25 Similarly, promoters and polyadenylation signals used must be functional within the cells of the vaccinated individual.

Examples of promoters useful to practice the present invention, especially in the production of a genetic vaccine for humans, include but are not limited to promoters from Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human Myosin, human Hemoglobin, human muscle creatine and human metallothionein.

Examples of polyadenylation signals useful to practice the present invention, especially in the production of a genetic vaccine for humans, include but are not limited to SV40 polyadenylation signals and LTR polyadenylation signals. In particular, the SV40 polyadenylation signal which is in pCEP4 plasmid (Invitrogen, San Diego Calif.), referred to as the SV40 polyadenylation signal, can be used.

In addition to the regulatory elements required for DNA expression, other elements may also be included in the DNA molecule. Such additional elements include enhancers. The enhancer may be selected from the group including but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from CMV, RSV and EBV.

Genetic constructs can be provided with mammalian origin of replication in order to maintain the construct extrachromosomally and produce multiple copies of the construct in the cell. Plasmids pCEP4 and pREP4 from Invitrogen (San Diego, Calif.) contain the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region which produces high copy episomal replication without integration.

An additional element may be added which serves as a target for cell destruction if it is desirable to eliminate cells receiving the genetic construct for any reason. A herpes thymidine kinase (tk) gene in an expressible form can be included in the genetic construct. When the construct is introduced into the cell, tk will be produced. The drug gangcyclovir can be administered to the individual and that drug will cause the selective killing of any cell producing tk. Thus, a system can be provided which allows for the selective destruction of vaccinated cells.

In order to be a functional genetic construct, the regulatory elements must be operably linked to the nucleotide sequence that encodes the target protein. Accordingly, it is necessary for the initiation and termination codons to be in frame with the coding sequence.

Open reading frames (ORFs) encoding the protein of interest and another or other proteins of interest may be introduced into the cell on the same vector or on different vectors. ORFs on a vector may be controlled by separate promoters or by a single promoter. In the latter arrangement, which gives rise to a polycistronic message, the ORFs will be separated by translational stop and start signals. The presence of an internal ribosome entry site (IRES) site between these ORFs permits the production of the expression product originating from the second ORF of

interest, or third, etc. by internal initiation of the translation of the bicistronic or polycistronic mRNA.

According to the invention, the genetic vaccine may be administered directly into the individual to be immunized or ex vivo into removed cells of the individual which are reimplanted after administration. By either route, the genetic material is introduced into cells which are present in the body of the individual. Routes of administration include, but are not limited to, intramuscular, intraperitoneal, intradermal, subcutaneous, intravenous, intraarterially, intraocularly and oral as well as transdermally or by inhalation or suppository. Preferred routes of administration include intramuscular, intraperitoneal, intradermal and subcutaneous injection. Genetic constructs may be administered by means including, but not limited to, traditional syringes, needleless injection devices, or microprojectile bombardment gene guns. Alternatively, the genetic vaccine may be introduced by various means into cells that are removed from the individual. Such means include, for example, ex vivo transfection, electroporation, microinjection and microprojectile bombardment. After the genetic construct is taken up by the cells, they are reimplanted into the individual. It is contemplated that otherwise non-immunogenic cells that have genetic constructs incorporated therein can be implanted into the individual even if the vaccinated cells were originally taken from another individual.

The genetic vaccines according to the present invention comprise about 1 nanogram to about 1000 micrograms of DNA. In some preferred embodiments, the vaccines contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the vaccines contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the vaccines contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the vaccines contain about 25 to about 250 micrograms of DNA. In some preferred embodiments, the vaccines contain about 100 micrograms DNA.

The genetic vaccines according to the present invention are formulated according to the mode of administration to be used. One having ordinary skill in the art can readily formulate a genetic vaccine that comprises a genetic construct. In cases where intramuscular injection is the chosen mode of administration, an isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include

gelatin and albumin. In some embodiments, a vaso-constriction agent is added to the formulation. The pharmaceutical preparations according to the present invention are provided sterile and pyrogen free.

Genetic constructs may optionally be formulated with one or more response enhancing agents such as: compounds which enhance transfection, i.e. transfecting agents; compounds which stimulate cell division, i.e. replication agents; compounds which stimulate immune cell migration to the site of administration, i.e. inflammatory agents; compounds which enhance an immune response, i.e. adjuvants or compounds having two or more of these activities.

In one embodiment, bupivacaine, a well known and commercially available pharmaceutical compound, is administered prior to, simultaneously with or subsequent to the genetic construct. Bupivacaine and the genetic construct may be formulated in the same composition. Bupivacaine is particularly useful as a cell stimulating agent in view of its many properties and activities when administered to tissue. Bupivacaine promotes and facilitates the uptake of genetic material by the cell. As such, it is a transfecting agent. Administration of genetic constructs in conjunction with bupivacaine facilitates entry of the genetic constructs into cells. Bupivacaine is believed to disrupt or otherwise render the cell membrane more permeable. Cell division and replication is stimulated by bupivacaine. Accordingly, bupivacaine acts as a replicating agent. Administration of bupivacaine also irritates and damages the tissue. As such, it acts as an inflammatory agent which elicits migration and chemotaxis of immune cells to the site of administration. In addition to the cells normally present at the site of administration, the cells of the immune system which migrate to the site in response to the inflammatory agent can come into contact with the administered genetic material and the bupivacaine. Bupivacaine, acting as a transfection agent, is available to promote uptake of genetic material by such cells of the immune system as well.

In addition to bupivacaine, mepivacaine, lidocaine, procains, carbocaine, methyl bupivacaine, and other similarly acting compounds may be used as response enhancing agents. Such agents acts a cell stimulating agents which promote the uptake of genetic constructs into the cell and stimulate cell replication as well as initiate an inflammatory response at the site of administration.

Other contemplated response enhancing agents which may function as transfecting agents and/or replicating agents and/or inflammatory agents and which may be administered include lectins, growth factors, cytokines and lymphokines

such as alpha-interferon, gamma-interferon, platelet derived growth factor (PDGF), gCSF, gMCSF, TNF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-6, IL-8, IL-10 and IL-12 as well as collagenase, fibroblast growth factor, estrogen, dexamethasone, saponins, surface active agents such as immune-stimulating complexes (ISCMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl Lipid A (MPL), muramyl peptides, quinone analogs and vesicles such as squalene and squalane, hyaluronic acid and hyaluronidase may also be used administered in conjunction with the genetic construct. In some embodiments, combinations of these agents are co-administered in conjunction with the genetic construct. In other embodiments, genes encoding these agents are included in the same or different genetic construct(s) for co-expression of the agents.

With respect to HIV Env, Gag, and Pol nucleotide sequences of the invention, particularly through genetic immunization, may be used as therapeutics or prophylactics in the treatment of AIDS or HIV infection. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that includes the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the (e.g., the concentration of the test compound which achieves a half-maximal inhibition of viral infection relative to the amount of the event in the absence of the test compound) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography (HPLC).

The compounds (for genetic immunization) of the invention may, further, serve the role of a prophylactic vaccine, wherein the host produces antibodies and/or CTL responses against HIV Env, Gag, and Pol which responses then preferably serve to neutralize HIV viruses by, for example, inhibiting further HIV infection.

5 Administration of the compounds of the invention as a prophylactic vaccine, therefore, would comprise administering to a host a concentration of compounds effective in raising an immune response which is sufficient to elicit antibody and/or CTL responses to HIV Env, Gag, and Pol and/or neutralize HIV, by, for example, inhibiting HIV ability to infect cells. The exact concentration will depend upon the
10 specific compound to be administered, but may be determined by using standard techniques for assaying the development of an immune response which are well known to those of ordinary skill in the art.

Env

To improve the immune response to native gp160 and to expose the core
15 protein for optimal antigen presentation and recognition, we have analyzed the immune response to modified forms of the protein. The role of conserved N-linked glycosylation sites has been studied, and analogues of fusion intermediates have been developed. Expression vectors with deletions in the cleavage site (C), the fusion peptide (F), and the interspace (I) between the two heptad repeats were
20 termed Δ CFL. Plasmid DNA vaccination has been a useful technology for the development and analysis of immunogens. This method of vaccination allows appropriate post-translational modification, proper intracellular trafficking, and antigen presentation. Direct injection of naked DNA either intramuscularly or intradermally in rodents induces immune responses, and the ability to easily modify
25 plasmid expression vectors to express different forms of HIV envelope proteins enables rapid and systematic testing of vaccine immunogens. In this disclosure, we have analyzed the immune response to modified Env candidates expressed in plasmids with modified codons to improve gene expression. Both antibody and CTL responses were analyzed after injection of plasmid DNA into muscle. A
30 modified gp140 DNA with improved ability to elicit antibody and CTL responses to HIV Env has now been identified that is envisioned as a prototype immunogen that can elicit broadly neutralizing antibody responses to HIV.

Exposing the Core Protein of Viral Membrane Fusion Proteins

Described herein are modified HIV envelope proteins that improve the immune response to native gp160 and expose the core protein for optimal antigen presentation and recognition. Weissenhorn et al., *Molecular Cell*, 2, 605-616, 1998

5 proposes a core protein as a model for a fusion intermediate of viral glycoproteins, where the glycoproteins are characterized by a central triple stranded coiled coil followed by a disulfide-bonded loop that reverses the chain direction and connects to an α helix packed antiparallel to the core helices, as, for example, in the case of Ebola Zaire GP2, Murine Moloney Leukemia virus (MuMoLv) 55-residue segment

10 of the TM subunit (Mo-55), low-pH-treated influenza HA2, protease resistant core of HIV gp41, and SIV gp41 (Figure A). Thus, the strategy for improving the immune response by exposing the protease resistant core of HIV gp41 extends to other viral membrane fusion proteins that are characterized by a central triple stranded coiled coil followed by a disulfide-bonded loop that reverses the chain

15 direction and connects to an α helix packed antiparallel to the core helices.

The present approach involves a series of internal mutations designed to replace the cleavage site (C), the fusion domain (F), and the interspace (I) between the two heptad repeats all on a backbone of COOH-terminal truncations to expose the core protein of the viral membrane fusion protein Env, based on modified gp140

20 as a prototype immunogen. By replacement is meant deletions, insertions, and/or substitutions of amino acid residues. In one embodiment, deletions are meant (i.e., amino acids are deleted to create the Δ CFI mutations).

In this embodiment, the Δ C mutation is intended to eliminate proteolysis by deleting the gp120/gp41 cleavage site that links the envelope covalently to the ectodomain by 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%,

25 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%,

30 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

In this embodiment, the Δ F mutation is intended to solubilize the molecule by deleting the fusion domain by 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%,

92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%,
 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%,
 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%,
 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42, 41%, 40%, 39%, 38%, 37%, 36%,
 5 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%,
 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%,
 6%, 5%, 4%, 3%, 2%, or 1%.

In this embodiment, the ΔI mutation is intended to stabilize oligomer
 formation by deleting the interspace between the two heptad repeats by 100%, 99%,
 10 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%,
 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%,
 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%,
 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42,
 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%,
 15 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%,
 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

In this embodiment, the COOH-terminal truncation is intended to reduce
 toxicity by deleting the cytoplasmic domain by 100%, 99%, 98%, 97%, 96%, 95%,
 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%,
 20 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%,
 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%,
 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42, 41%, 40%, 39%, 38%,
 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%,
 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%,
 25 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

In this embodiment, optionally, the COOH-terminal truncation is extended
 so as to solubilize the molecule by deleting the transmembrane domain by 100%
 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%,
 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%,
 30 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%,
 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%,
 43%, 42, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%,
 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%,
 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

Amino acid substitutions may encompass those of a conserved or non-conserved nature. Presumably, a non-conserved substitution of a domain would act like a deletion of the domain. Conserved amino acid substitutions constitute switching one or more amino acids with amino acids of similar charge, size, and/or hydrophobicity characteristics. Non-conserved amino acid substitutions constitute switching one or more amino acids with amino acids of dissimilar charge, size, and/or hydrophobicity characteristics. The families of amino acids include the basic amino acids (lysine, arginine, histidine); the acidic amino acids (aspartic acid, glutamic acid); the non-polar amino acids (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); the uncharged polar amino acids (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine); and the aromatic amino acids (phenylalanine, tryptophan, and tyrosine). One or more substitutions may be introduced to achieve the ΔC mutation intended to eliminate proteolysis by acting like a deletion of the gp120/gp41 cleavage site to link the envelope covalently to the ectodomain, the ΔF mutation intended to solubilize the molecule by acting like a deletion of the fusion domain, the ΔI mutation intended to stabilize oligomer formation by acting like a deletion of the interspace between the two heptad repeats, the COOH-terminal truncation intended to reduce toxicity by acting like a deletion of the cytoplasmic domain, and, optionally, the COOH-terminal truncation extended so as to solubilize the molecule by acting like a deletion of the transmembrane domain.

Amino acid insertions may constitute single amino acid residues or stretches of residues. The insertions may be made at the carboxy or amino terminal end of a domain, as well as at a position internal to the domain. Such insertions will generally range from 2 to 15 amino acids in length. One or more insertions may be introduced to achieve the ΔC mutation intended to eliminate proteolysis by acting like a deletion of the gp120/gp41 cleavage site to link the envelope covalently to the ectodomain, the ΔF mutation intended to solubilize the molecule by acting like a deletion of the fusion domain, the ΔI mutation intended to stabilize oligomer formation by acting like a deletion the interspace between the two heptad repeats, the COOH-terminal truncation intended to reduce toxicity by acting like a deletion of the cytoplasmic domain, and, optionally, the COOH-terminal truncation extended so as to solubilize the molecule by acting like a deletion of the transmembrane domain.

The nucleic acids of the present invention are optionally DNA, RNA, or mRNA. Most typically, the nucleic acids are provided by recombinantly making a DNA, which is expressed in a cell as RNA and/or as mRNA. Given the strategy for making the nucleic acids of the present invention, one of skill can construct a variety of clones containing functionally equivalent nucleic acids. Cloning methodologies to accomplish these ends, and sequencing methods to verify the sequence of nucleic acids are well known in the art. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are found in Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology*, volume 152, Academic Press, Inc., San Diego, Calif. (Berger); Sambrook, et al. (1989) *Molecular Cloning--A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, N.Y., (Sambrook); and *Current Protocols in Molecular Biology*, F. M. Ausubel, et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement) (Ausubel). Product information from manufacturers of biological reagents and experimental equipment also provide information useful in known biological methods. Such manufacturers include the SIGMA chemical company (Saint Louis, Mo.), R&D systems (Minneapolis, Minn.), Pharmacia LKB Biotechnology (Piscataway, N.J.), CLONTECH Laboratories, Inc. (Palo Alto, Calif.), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, Wis.), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersburg, Md.), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen, San Diego, Calif., and Applied Biosystems (Foster City, Calif.), as well as many other commercial sources known to one of skill.

The nucleic acid compositions of this invention, whether RNA, cDNA, mRNA, genomic DNA, or a hybrid of the various combinations, are isolated from biological sources or synthesized in vitro. The nucleic acids of the present invention are present in transformed or transfected whole cells, in transformed or transfected cell lysates, or in a partially purified or substantially pure form.

In vitro amplification techniques suitable for amplifying sequences to provide a nucleic acid or for subsequent analysis, sequencing or subcloning are known. Examples of techniques sufficient to direct persons of skill through such in vitro amplification methods, including the polymerase chain reaction (PCR) the

ligase chain reaction (LCR), Q β -replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA) are found in Berger, Sambrook, and Ausubel, as well as Mullis, et al., (1987) U.S. Pat. No. 4,683,202; PCR Protocols A Guide to Methods and Applications (Innis, et al. eds) Academic Press Inc. San Diego, Calif. 5 (1990) (Innis); Arnheim & Levinson (Oct. 1, 1990) C&EN 36-47; The Journal Of NIH Research (1991) 3:81-94; Kwoh, et al., Proc. Natl. Acad. Sci. USA, 86:1173 (1989); Guatelli, et al., Proc. Natl. Acad. Sci. USA, 87:1874 (1990); Lomell, et al., J. Clin. Chem., 35:1826 (1989); Landegren, et al., Science, 241:1077-1080 (1988); Van Brunt, Biotechnology, 8:291-294 (1990); Wu and Wallace, Gene, 4:560 (1989); 10 Barringer, et al., Gene, 89:117 (1990), and Sooknanan and Malek, Biotechnology, 13:563-564 (1995). Improved methods of cloning in vitro amplified nucleic acids are described in Wallace, et al., U.S. Pat. No. 5,426,039. Improved methods of amplifying large nucleic acids (up to 40 kb) are summarized in Cheng, et al., Nature, 369:684-685 (1994) and the references therein. One of skill will appreciate that 15 essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, Ausubel, Sambrook, Innis, and Berger, all supra.

One of skill will recognize many ways of generating alterations in a given nucleic acid construct. Such well-known methods include site-directed mutagenesis, 20 PCR amplification using degenerate oligonucleotides, exposure of cells containing the nucleic acid to mutagenic agents or radiation, chemical synthesis of a desired oligonucleotide (e.g., in conjunction with ligation and/or cloning to generate large nucleic acids) and other well-known techniques. See, Giliman and Smith, Gene 8:81-97 (1979), Roberts, et al., Nature, 328:731-734 (1987) and Sambrook, Innis, 25 Ausubel, Berger, and Mullis (all supra).

Most modifications to nucleic acids are evaluated by routine screening techniques in suitable assays for the desired characteristic. For instance, changes in the immunological character of encoded polypeptides can be detected by an appropriate immunological assay. For instance, changes in the cellular 30 immunological character of the polypeptide can be detected by an appropriate antibody or CTL assay. Modifications of other properties such as nucleic acid hybridization to a complementary nucleic acid, redox or thermal stability of encoded proteins, hydrophobicity, susceptibility to proteolysis, or the tendency to aggregate are all assayed according to standard techniques.

A wide variety of formats and labels are available and appropriate for detection of polypeptide sequences. These include analytic biochemical methods such as spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, and various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), western blot assays, immunofluorescent assays, and the like. Several commercially available ELISA assays for the detection of retroviral components, including Env domains, are available, allowing one of skill to detect Env in biological samples.

Similarly, the detection of the nucleic acids of the present invention proceeds by well known methods such as Southern analysis, northern analysis, gel electrophoresis, PCR, radiolabeling and scintillation counting, and affinity chromatography. Many assay formats are appropriate, including those reviewed in Tijssen (1993) *Laboratory Techniques in biochemistry and molecular biology--hybridization with nucleic acid probes parts I and II*, Elsevier, New York and Choo (ed) (1994) *Methods In Molecular Biology Volume 33--In Situ Hybridization Protocols*, Humana Press Inc., New Jersey (see also, other books in the *Methods in Molecular Biology* series); see especially, Chapter 21 of Choo (id.) "Detection of Virus Nucleic Acids by Radioactive and Nonisotopic in Situ Hybridization". Finally, PCR is also routinely used to detect nucleic acids in biological samples (see, Innis, *supra*, for a general description of PCR techniques).

In one preferred embodiment, antibodies are used to detect polypeptide sequences. Methods of producing polyclonal and monoclonal antibodies are known to those of skill in the art, and many anti-HIV antibodies are available. See, e.g., Coligan (1991) *Current Protocols in Immunology*, Wiley/Greene, NY; and Harlow and Lane (1989) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, NY; Stites, et al. (eds.) *Basic and Clinical Immunology* (4th ed.), Lange Medical Publications, Los Altos, Calif., and references cited therein; Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2d ed.), Academic Press, New York, N.Y.; and Kohler and Milstein, *Nature*, 256:495-497 (1975). Other suitable techniques for antibody preparation include selection of libraries of recombinant antibodies in phage or similar vectors. See, Huse, et al., *Science*, 246:1275-1281 (1989); and Ward, et al., *Nature*, 341:544-546 (1989). Specific monoclonal and

polyclonal antibodies and antisera will usually bind with a KD of at least about 0.1 mM, more usually at least about 1 μ M, preferably at least about 0.1 μ M or better, and most typically and preferably, 0.01 μ M or better.

Development of HIV Env Vectors

5 To develop Env glycoprotein variants that might effectively induce humoral and cellular immunity, a series of plasmid expression vectors were generated (Fig. I). HIV Env is encoded by nucleic acid sequences that contain RNA structures that limit gene expression (Andre S et al, 1998, *J Virol*, 72:1497-503; Qiu JT et al., 1999, *J Virol*, 73:9145-52; Rana TM et al., 1999, *Arch Biochem Biophys*, 365:175-85; 10 Roebuck KA, Saifuddin M, 1999, *Gene Expr*, 8:67-84; Romano G, et al., 1999, *J Cell Biochem*, 75:357-68; Schneider R et al., 1997, *J Virol*, 71:4892-903). These vectors were therefore synthesized using codons found in human genes that allow these structures to be eliminated without affecting the amino acid sequence. Full-length HIV Env (gp160) was highly expressed in the absence of HIV accessory 15 proteins at levels ≥ 10 -fold higher than Rev-dependent viral gp 160 in transfected 293 cell by Western blot analysis (Fig. IIA), and the relevant mutant proteins were detected at the expected apparent molecular weights (Fig. IIC-D). As might be anticipated, gp160 expressed from the synthetic gene was not efficiently processed in transfected 293 cells, presumably because over-expression of gp160 saturates the 20 cellular proteases responsible for cleavage. (Binley JM et al., 2000, *J. Virol*, 74:627-643.) Synthetic HIV gp160 induced toxicity in transfected cells, with cell rounding and detachment evident within 48 hours (Fig. IIIA vs B). This cytotoxicity was reduced by elimination of the COOH-terminal cytoplasmic domain. Env protein that terminated at amino acid 752 (gp150) was less cytotoxic than gp160, while the 25 shorter proteins (gp145 and gp140) produced little or no effect (Fig. IIIC, D, and E, respectively).

To alter Env immunogenicity, two different approaches were explored. First the effects of glycosylation on cellular and humoral immunity were evaluated by analysis of mutants in which conserved N-linked glycosylation sites were eliminated 30 by site-directed mutagenesis. Two sets of mutations were introduced into both gp160 and gp150 (Fig. I). The first set included eleven potential sites (Δ gly11), and the second set included an additional 6 sites downstream (Δ gly17). Expression studies showed that the glycosylation mutants were efficiently expressed, and the

glycosylation mutant protein was appropriately reduced in size compared to wild type gp160 or gp150, consistent with reduced N-linked glycosylation (Fig IIC).

5 The second approach involved a series of internal deletions designed to stabilize and expose functional domains of the protein that might be present in an extended helical structure prior to the formation of the six-member coiled-coil structure in the hairpin intermediate (Chan DC et al., 1997, Cell, 89:263-73; Weissenhorn W et al., 1997, Nature, 387:426-30). To generate this putative pre-hairpin structure, the cleavage site was removed to prevent the proteolytic processing of the envelope and stabilize the protein by linking it covalently to the gp41 extracellular and/or transmembrane domain. To reduce toxicity and enhance stability, the fusion peptide domain was deleted. The heptad repeats in the envelope protein are important tertiary structure domains involved in the ability of the envelope protein to form trimers (Lu M, Kim PS, 1997, J Biomol Struct Dyn, 15:465-71). The sequence between the heptad repeats was removed to stabilize the formation of trimers and eliminate formation of the hairpin intermediate. These Δ CFI deletions were introduced into full-length gp160 and COOH-terminal truncation mutants. Though cells transfected with vectors encoding gp140 Δ CFI, gp145 Δ CFI and gp160 readily expressed these proteins (Fig. IID), only gp140 Δ CFI, which lacks the transmembrane domain, was readily detected in the supernatant (Fig. IV), indicating that it can give rise to soluble antigen.

Immunogenicity of Env Mutants After DNA Vaccination

The ability of these Env proteins to elicit an immune response was determined in mice by injection with these plasmid DNA expression vectors. Antibody responses were monitored by the ability of antisera from injected mice to immunoprecipitate wild type gp160 from cell lysates by SDS-PAGE and Western blotting (Fig. V). In some cases, antibody reactivity was also confirmed by immunofluorescence. To quantitate the antibody response, immunoprecipitation followed by Western blotting with different dilutions of immunized mouse sera was tested. The intensity of the gp160 band was determined by densitometry and standardized relative to a positive control sera used to normalize data between experiments. The approximately linear dose response of gp160 intensity with serum dilution allowed quantification of the anti-gp160 antibody response in mouse sera. Data from immunized mice showed that none of the wild type Env proteins, neither the gp160, gp150, gp145, nor gp140 COOH-terminal truncations generated

consistently high antibody responses (Fig. VA). Of these proteins, gp140 was somewhat more effective than gp160 in generating antibody responses but remained low and inconsistent. Immunization with vectors designed to express glycosylation deficient envelope proteins also did not improve the humoral response. In contrast, the Δ CFI mutants in the Env truncation vectors substantially increased anti-gp160 antibody response (Fig. VA; gp145, gp140, and gp128). The longer Δ CFI Env proteins (gp160 and gp150) did not show comparable enhancement of this response. The gp140 (Δ CFI) provided more consistent and a greater increase in antibody response than gp128 Δ CFI (Fig VB). In all cases, these vectors that encoded gp140 Δ CFI, but not wild type gp140, induced antibodies that were reactive to native gp160 (Fig. VC).

To determine whether these modifications of Env adversely affected CTL responses, spleen cells from immunized mice were tested for their ability to lyse relevant target cells. All mice immunized with codon-altered Env vectors, including COOH-terminal deletion mutations (Fig. VIA) or glycosylation mutants (Fig. VIB), elicited strong CTL responses directed to cell lines pulsed with HIV Env peptides. These findings were also confirmed using stably transfected cells that expressed Env. Importantly, gp140 (Δ CFI), which elicited increased antibody responses relative to the comparable wild typ Env, readily induced CTL responses to native Env, as did other Δ CFI mutants (Fig. VIC). Addition of anti-CD8 antibody inhibited cytolytic activity, as did depletion using magnetic beads coupled with anti-CD8 antibody, thus confirming a cytotoxic T cell response to these immunogens after genetic immunization (Fig. VID). This response was detectable for at least six months after immunization.

Glycosylation and Δ CFI HIV Env Mutants

To develop DNA vaccine candidates for HIV, we developed a series of synthetic genes designed to express HIV Env mutants in human cells. In the absence of HIV regulatory proteins, these codon-altered envelope protein genes expressed well in human cells. Like other DNA vaccines, immunization with these vectors elicited strong CTL responses in mice, and antibody responses were not robust in mice immunized with wild type Env expression vectors. Mutations in highly conserved N-linked glycosylation sites did not significantly alter humoral or cellular immune response to native Env. In contrast, a mutant Env with deletions in the cleavage site, fusion domain, and a region between the heptad repeats elicited a

more potent humoral immune response and retained its ability to stimulate Env-specific CTL.

Recent reports suggest that gp160 forms trimers *in vivo* and the domain required for trimer formation resides in the ectodomain of the gp41 (Yang X et al., 2000, J Virol, 74:5716-25). Such trimeric forms of HIV envelope protein are likely to present different epitopes to the immune system compared to monomeric gp120. In addition to the linear epitopes in the envelope, this trimeric structure is likely to expose conformational epitopes important for B cell triggering of a relevant antibody response. In this regard, gp140 (Δ CFI), which induced the greatest antibody response, is released in a soluble form (Fig. IVB). In contrast, wild type Env did not elicit high titer antibody responses. The toxicity of Env in mammalian cells has been seen and could limit both the amount and duration of envelope protein expression *in vivo* that would affect immunogenicity. The envelope is also heavily glycosylated, and removal of partial or complete gp120 glycosylation sites has resulted in higher titers of strain-specific neutralizing antibody responses to mutant SIVs in monkeys (Binley JM et al., 1998, AIDS Res Hum Retroviruses, 14 :191-8; Reitter JN et al., 1998, Nat Med, 4:679-84). Though it seemed reasonable that deglycosylation would reveal epitopes otherwise masked in the native protein, we did not observe enhanced immune reactivity by DNA vaccination using different glycosylation site mutants, both in gp160 and gp150. This difference with the previous study is likely due to the fact that DNA vaccination rather than viral infection was utilized for immunization. Though glycosylation mutants are unlikely to prove helpful with this former method of immunization, we envision that modification of glycosylation sites will be effective with other vectors or adjuvants.

HIV-1 Env is proteolytically cleaved by a cellular convertase into gp120 and gp41 (Eckert DM et al., 1999, Cell, 99:103-15). The gp41 subunit is composed of cytoplasmic, transmembrane, and ectodomain segments. The role of the ectodomain of the envelope in membrane fusion, particularly its hydrophobic glycine-rich fusion peptide, is well established. Two regions with heptad coiled-coil repeats in the ectodomain of gp41 are involved in viral fusion (Eckert DM et al., 1999, Cell, 99:103-15; Weissenhorn W et al., 1996, EMBO J, 15:1507-14). Upon fusion, these two alpha helices, connected via a disulfide-stabilized loop (Gallaher WR et al., 1989, AIDS Res Hum Retroviruses, 5:431-40; Kent KA, Robinson J, 1996, AIDS, 10 Suppl A:S107-S114; Sattentau QJ et al., 1995, Virology, 206:713-7), presumably

undergo a transient conformational change to a fusion active state. These changes allow the formation of a six-member helical hairpin intermediate structure that presumably exposes the fusion peptide at the NH₂-terminus of gp41, allowing fusion to the target cell membrane (Binley J, Moore JP, [published erratum appears in Nature 1997 Sep 11;389(6647):131], 1997, Nature, 387 :346-8; LaCasse R et al., 1999, Science, 283:357-62). The Δ CFI mutation was intended to eliminate cleavage of gp140, remove the unstable hydrophobic region and stabilize oligomer formation. Though detailed structural data is not yet available on this protein, these mutations apparently stabilize Env in a conformation that elicits both humoral and cellular immune responses. For example, the neutralizing epitope in the ectodomain of gp41 (Muster T et al., 1994, J Virol, 68:4031-4) is present in the series of deletions and truncations of the envelope and gp140 (Δ CFI) is reactive with the 2F5 neutralizing monoclonal antibody that binds to this epitope. Importantly, these immunogens also induced CTL responses to Env. Though gp128 (Δ CFI) induced slightly more potent CTL activity, gp140 (Δ CFI) was better able to elicit such responses, both to peptide-pulsed cells and stably transduced target cells. Thus the enhanced humoral immune response introduced by this vaccine candidate did not appear to diminish the CTL response. Taken together, these results indicate that gp140 Δ CFI serves as an improved immunogen that can more effectively elicit an antibody response against the envelope by DNA vaccination while preserving its ability to induce a CTL response.

Gag and Pol

In this disclosure, we have prepared synthetic HIV-1 B clade Gag and Pol expression vectors that are based on human (h) codon usage. These vectors encode hGag-Pol and its derivatives, hGag, hPol and an hGag-Pol fusion protein. The synthetic Gag-Pol genes show little nucleotide homology to HIV-1 but are the same in protein sequence. The modified Gag-Pol genes were subcloned into a eukaryotic plasmid expression vector for expression and DNA immunization studies. Synthetic Gag-Pol genes allowed high level Rev-independent expression of HIV-1 Gag-Pol precursor proteins in human and mouse cell lines and induced significant cellular and humoral responses in mice. The Gag-Pol fusion protein induced the broadest responses to Gag and Pol determinants and thus is envisioned as a prototype immunogen that maximizes epitope presentation.

Eliminating The Frame Shift Site To Create Viral Polyproteins

Described herein are HIV Gag-Pol fusion proteins encoded by a continuous open reading frame so to improve the immune response to native Gag and Pol. In some viruses, translational frame shifting is exploited during protein synthesis. Specific sequences in the RNA are required for the frame shifting. The viral RNA sequences cause ribosomal slippage so that viral proteins are produced in non-equivalent ratios. For example, during translation in HIV, the ribosomes shift reading frames to synthesize Gag precursor protein and the gag-pol fusion protein in a 20:1 ratio. The strategy here is to maximize epitope presentation by transcribing an immunogen from a continuous open reading frame by eliminating the frame shift site. Thus, the strategy for improving the immune response by the use of a HIV Gag-Pol fusion protein encoded by a continuous open reading frame extends to other viral proteins that are produced in non-equivalent ratios by virtue of translational frame shifting.

The present invention involves HIV Gag-Pol fusion proteins encoded by a single continuous open reading frame due to mutation of the frame shift site. The frame shift site is mutated by deletions, insertions, and/or substitutions of nucleotides to create a single continuous open reading frame. In one embodiment, deletions are meant (i.e., nucleotides are deleted to create the same open reading frame).

The frame shift site is a mutated frame shift to create a single continuous open reading frame. For example, a set of similar retroviral gag-pol frame shift sites are optionally made for a given fusion protein, for example, by synthesizing different gag-pol frame shift regions and cloning the sequences appropriately, or by site-directed mutagenesis of a given frame shift clone. The efficacy of the frame shift sites are assessed by measuring the production of the fusion protein. The sequence that shows the highest level of expression is a "optimized" frame shift mutation for the set assessed. Alternatively, where a particular level of expression is desired, a frame shift site from a particular set of possible frame shift sites which is closest to the desired activity level is considered to be "optimized."

Although a full length Gag sequence is preferred for use in the fusion protein of the present invention, Gag is optionally deleted of subsequences without negating a polyepitope response. For example, regions of the matrix protein (p17), regions of the capsid protein (p24), regions of p2, regions of the nucleocapsid protein (p7), regions of p1, and regions of p6 can be deleted while preserving the polyepitope

response. Alternatively, regions of the matrix protein (p17), regions of the capsid protein (p24), regions of p2, regions of the nucleocapsid protein (p7), regions of p1, and regions of p6 can be substituted while preserving the polyepitope response .
Alternatively, regions of the matrix protein (p17), regions of the capsid protein (p24), regions of p2, regions of the nucleocapsid protein (p7), regions of p1, and regions of p6 can be interrupted by insertions while preserving the polyepitope response. Optionally, regions of the matrix protein (p17), regions of the capsid protein (p24), regions of p2, regions of the nucleocapsid protein (p7), regions of p1, and regions of p6 can be mutated to inactivate these proteins.

Likewise, a full length Pol sequence is preferred for use in the fusion protein of the present invention, and Pol is optionally deleted of subsequences without negating a polyepitope response. For example, regions of the protease protein, regions of the reverse transcriptase protein, and regions of the integrase protein can be deleted while preserving the polyepitope response. Alternatively, regions of the protease protein, regions of the reverse transcriptase protein, and regions of the integrase protein can be substituted while preserving the polyepitope response. Alternatively, regions of the protease protein, regions of the reverse transcriptase protein, and regions of the integrase protein can be interrupted by insertions while preserving the polyepitope response. Optionally, regions of the protease protein, regions of the reverse transcriptase protein, and regions of the integrase protein can be mutated to inactivate these enzymes.

In other embodiments, the Gag-Pol fusion proteins are co-expressed with other proteins, either as fusion proteins or separately, preferably with Env sequence proteins, such as the modified HIV Env Δ CFI proteins described herein.

In another aspect, the invention involves chimeric nucleic acid molecules. The chimeric nucleic acid molecules of the invention typically have a retroviral gag nucleic acid sequence and a retroviral pol nucleic acid sequence in the same open reading frame due to mutation of the frame shift site. The continuous open reading frame encodes a fusion protein such as those described above.

The chimeric nucleic acids of the present invention are optionally DNA, RNA, or mRNA. Most typically, the chimeric nucleic acids are provided by recombinantly making a DNA, which is expressed in a cell as RNA and/or as mRNA. Given the strategy for making the chimeric nucleic acids of the present invention, one of skill can construct a variety of clones containing functionally

equivalent nucleic acids. Cloning methodologies to accomplish these ends, and sequencing methods to verify the sequence of nucleic acids are well known in the art. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are found in

5 Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, volume 152, Academic Press, Inc., San Diego, Calif. (Berger); Sambrook, et al. (1989) Molecular Cloning--A Laboratory Manual (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, N.Y., (Sambrook); and Current Protocols in Molecular Biology, F. M. Ausubel, et al., eds., Current

10 Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement) (Ausubel). Product information from manufacturers of biological reagents and experimental equipment also provide information useful in known biological methods. Such manufacturers include the SIGMA chemical company (Saint Louis, Mo.), R&D systems (Minneapolis, Minn.),

15 Pharmacia LKB Biotechnology (Piscataway, N.J.), CLONTECH Laboratories, Inc. (Palo Alto, Calif.), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, Wis.), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersburg, Md.), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen, San Diego, Calif., and Applied Biosystems (Foster City, Calif.), as well as many other commercial sources known to one of skill.

20

The chimeric nucleic acid compositions of this invention, whether RNA, cDNA, mRNA, genomic DNA, or a hybrid of the various combinations, are isolated from biological sources or synthesized in vitro. The chimeric nucleic acids of the present invention are present in transformed or transfected whole cells, in

25 transformed or transfected cell lysates, or in a partially purified or substantially pure form.

In vitro amplification techniques suitable for amplifying sequences to provide a nucleic acid or for subsequent analysis, sequencing or subcloning are known. Examples of techniques sufficient to direct persons of skill through such in vitro amplification methods, including the polymerase chain reaction (PCR) the

30 ligase chain reaction (LCR), QB-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA) are found in Berger, Sambrook, and Ausubel, as well as Mullis, et al., (1987) U.S. Pat. No. 4,683,202; PCR Protocols A Guide to Methods and Applications (Innis, et al. eds) Academic Press Inc. San Diego, Calif.

(1990) (Innis); Arnheim & Levinson (Oct. 1, 1990) C&EN 36-47; The Journal Of NIH Research (1991) 3:81-94; Kwoh, et al., Proc. Natl. Acad. Sci. USA, 86:1173 (1989); Guatelli, et al., Proc. Natl. Acad. Sci. USA, 87:1874 (1990); Lomell, et al., J. Clin. Chem., 35:1826 (1989); Landegren, et al., Science, 241:1077-1080 (1988);
5 Van Brunt, Biotechnology, 8:291-294 (1990); Wu and Wallace, Gene, 4:560 (1989); Barringer, et al., Gene, 89:117 (1990), and Sooknanan and Malek, Biotechnology, 13:563-564 (1995). Improved methods of cloning in vitro amplified nucleic acids are described in Wallace, et al., U.S. Pat. No. 5,426,039. Improved methods of amplifying large nucleic acids (up to 40 kb) are summarized in Cheng, et al., Nature,
10 369:684-685 (1994) and the references therein. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, Ausubel, Sambrook, Innis, and Berger, all supra.

One of skill will recognize many ways of generating alterations in a given
15 nucleic acid construct. Such well-known methods include site-directed mutagenesis, PCR amplification using degenerate oligonucleotides, exposure of cells containing the nucleic acid to mutagenic agents or radiation, chemical synthesis of a desired oligonucleotide (e.g., in conjunction with ligation and/or cloning to generate large nucleic acids) and other well-known techniques. See, Giliman and Smith, Gene
20 8:81-97 (1979), Roberts, et al., Nature, 328:731-734 (1987) and Sambrook, Innis, Ausubel, Berger, and Mullis (all supra).

Most modifications to nucleic acids are evaluated by routine screening techniques in suitable assays for the desired characteristic. For instance, changes in the immunological character of encoded polypeptides can be detected by an
25 appropriate immunological assay. For instance, changes in the cellular immunological character of the polypeptide can be detected by an appropriate antibody or CTL assay. Modifications of other properties such as nucleic acid hybridization to a complementary nucleic acid, redox or thermal stability of encoded proteins, hydrophobicity, susceptibility to proteolysis, or the tendency to aggregate
30 are all assayed according to standard techniques.

A wide variety of formats and labels are available and appropriate for detection of fusion protein sequences. These include analytic biochemical methods such as spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography
35 (TLC), hyperdiffusion chromatography, and the like, and various immunological

methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), western blot assays, immunofluorescent assays, and the like. Several commercially available ELISA assays for the detection of retroviral components, including Env domains, are available, allowing one of skill to detect Env in biological samples.

Similarly, the detection of the chimeric nucleic acids of the present invention proceeds by well known methods such as Southern analysis, northern analysis, gel electrophoresis, PCR, radiolabeling and scintillation counting, and affinity chromatography. Many assay formats are appropriate, including those reviewed in Tijssen (1993) *Laboratory Techniques in biochemistry and molecular biology--hybridization with nucleic acid probes* parts I and II, Elsevier, New York and Choo (ed) (1994) *Methods In Molecular Biology Volume 33--In Situ Hybridization Protocols*, Humana Press Inc., New Jersey (see also, other books in the *Methods in Molecular Biology* series); see especially, Chapter 21 of Choo (id.) "Detection of Virus Nucleic Acids by Radioactive and Nonisotopic in Situ Hybridization". Finally, PCR is also routinely used to detect nucleic acids in biological samples (see, Innis, *supra*, for a general description of PCR techniques).

In one preferred embodiment, antibodies are used to detect polypeptide sequences. Methods of producing polyclonal and monoclonal antibodies are known to those of skill in the art, and many anti-HIV antibodies are available. See, e.g., Coligan (1991) *Current Protocols in Immunology*, Wiley/Greene, NY; and Harlow and Lane (1989) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, NY; Slites, et al. (eds.) *Basic and Clinical Immunology* (4th ed.), Lange Medical Publications, Los Altos, Calif., and references cited therein; Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2d ed.); Academic Press, New York, N.Y.; and Kohler and Milstein, *Nature*, 256:495-497 (1975). Other suitable techniques for antibody preparation include selection of libraries of recombinant antibodies in phage or similar vectors. See, Huse, et al., *Science*, 246:1275-1281 (1989); and Ward, et al., *Nature*, 341:544-546 (1989). Specific monoclonal and polyclonal antibodies and antisera will usually bind with a KD of at least about 0.1 mM, more usually at least about 1 μ M, preferably at least about 0.1 μ M or better, and most typically and preferably, 0.01 μ M or better.

Expression of Synthetic HIV-1 Gag and Pol Genes

Four synthetic HIV-1 Gag-and/or Pol expression vectors, hGag-Pol, hGag-Pol Δ FS Δ Pr, hPol and hGag genes were prepared (Fig. VII). To confirm expression, the synthetic or viral Gag-Pol genes were transiently transfected into 293T cells, a human kidney-derived cell line. When cell lysates were analyzed by immunoblotting with human anti-HIV-1 IgG (Fig. VIIIA), monoclonal anti-p24 (Fig. VIIIB), and rabbit anti-RT (Fig. VIIC), Gag p55, Pol p110 and Gag-Pol p160 precursor proteins were detected in hGag, hPol, and hGag-Pol fusion plasmids transfected 293T cells, as was expected. Mature virion proteins, p24 and RTp66, were detected in the hGag-Pol gene transfected cells (Figs. VIIIA, B and C). This might be a result of the activation of protease inside cells which was itself a result of the high-level expression of Gag and Gag-Pol protein (Karacostas, V et al., 1993, Virology, 193:661-671). The expression of Gag precursor proteins from codon-altered vectors was ≥ 10 -fold higher than viral Gag-Pol (Fig. VIII), determined by quantitative phosphorimaging. The level of accumulated Gag-Pol fusion protein was 100-fold higher in cells transfected with hGag-Pol compared to viral Gag-Pol. Virus-like particles were released from the hGag gene transfected cells (Fig. IX), detected by transmission electron microscopy. Though such particles were observed at a lower frequency with hGag-Pol, no particles were seen in cells transfected with hGag-Pol Δ FS Δ Pr or hPol vectors. Stable expression of HIV-1 Gag and Pol proteins from codon-optimized genes in mouse CT26 and BH10ME cells was also observed (Fig. VIID).

Induction of HIV-1 Gag and Pol CTL Responses in Mice by DNA Vaccination

To evaluate the cellular immune response to HIV-1 Gag and Pol proteins, Balb/C female mice were injected intramuscularly with the eukaryotic expression vector plasmids containing the codon-optimized genes. Two weeks after the final vaccination, splenocytes were harvested from the immunized mice and sensitized with either Gag or Pol peptide-pulsed naïve mouse splenocytes. One week later, CTL responses were analyzed using a 5-hour chromium release assay.

CTL responses specific to HIV-1 Gag and/or Pol were first analyzed using Gag or Pol peptide-pulsed BC10ME cells, or mouse fibrosarcoma cell lines derived from B/C-N cells (Collins, JL et al., 1981, J Exp. Med., 153:89-106). Immunization with hGag, hGag-Pol Δ FS Δ Pr or hGag-Pol genes induced comparably strong CTL responses specific to Gag (Fig. XA); however, after immunization with hPol, hGag-

Pol Δ FS Δ Pr or hGag-Pol genes, only the fusion protein, hGag-Pol Δ FS Δ Pr, and hPol to a lesser extent, elicited a marked CTL response to Pol (Fig. XB). To confirm that the specific killing in the CTL assays was induced by CD8⁺ cytotoxic T lymphocytes, CD4⁺ or CD8⁺ cells were depleted from sensitized splenocytes by Dynal beads (Dynal, Inc., Lake Success, NY). Depletion of CD8⁺ cells abolished the specific lysis in the hGag-Pol Δ FS Δ Pr gene-immunized mice, while depletion of CD4⁺ had little effect on lysis (Fig. XC), suggesting that CD8⁺ lymphocytes were responsible for specific cytotoxicity.

The responses were further analyzed and confirmed with the hGag or hPol gene transduced syngeneic CT26 and BC10ME cell lines. Responses to Gag in the mice immunized with the hGag, hGag-Pol Δ FS Δ Pr or hGag-Pol genes were similar when peptide-pulsed cells were used as targets in the CTL assay (Fig. XIA). Mice immunized with the hPol gene generated a specific response to HIV-1 Pol on BC10ME cell lines stably expressing Pol as target cells (Fig. XIB). The same results have been observed with CT26 cell lines. These stably transfected cell lines were therefore more sensitive as target cells than peptide-pulsed cells in the Pol CTL assays.

Antibody Response in the Immunized Mice

Sera from mice immunized with different plasmids was analyzed with a p24 ELISA. hGag immunized mice demonstrated the highest p24 antibody titers (Fig. XIIA). Unexpectedly, hGag-Pol virus-like particles elicited the lowest levels of p24 antibody. Similar results were observed by Western blotting with pooled sera (Fig. XIIB). The HIV-1 Pol specific antibodies were not detected by a commercially available Western blotting kit (Fig. XIIB), but antibodies to Pol were detected in mice immunized with hPol and hGag-Pol Δ FS Δ Pr with a more sensitive method, IP/Western blotting (Fig. XIIC). Presumably, this assay is more sensitive and better able to detect native conformational epitopes. Though such antibodies were found in mice immunized with Pol and Gag-Pol fusion proteins in this assay, minimal response was detected in the mice immunized with hGag-Pol. Though both immunogens elicited similar Gag responses, the Gag-Pol fusion protein was therefore more effective in the stimulation of CTL and antibody responses to Pol.

HIV Gag-Pol Fusion Proteins

In this disclosure, HIV-1 B-clade Gag and Pol genes were modified to increase Rev-independent expression of HIV-1 Gag-Pol proteins. This modification

allowed synthesis of HIV Gag and Pol, as well as fusion proteins at levels 10- to 100-fold higher than the corresponding Rev-dependent viral gene in the absence of Rev and RRE elements. These viral proteins were recognized by standard polyclonal and monoclonal antibodies (Fig. VIII), and immature VLP were produced and released from 293T cells transfected with hGag (Fig. IX).

The immune response induced by Rev-independent Gag-Pol was directed against both Gag and Pol determinants. The hGag, hGag-Pol fusion and hGag-Pol all induced strong CTL responses specific for Gag in mice immunized with plasmid DNA, but a significant Pol response was elicited only in the mice immunized with the hGag-Pol Δ FS Δ Pr or Pol alone. Because immunization with hGag-Pol gene failed to induce detectable cellular or humoral responses to HIV-1 Pol protein, these findings indicate that the Gag-Pol fusion protein induces a broader range of responses and allows delivery of an immunogen with a larger number of epitopes in a single continuous open reading frame. During viral replication, viral gag-pol produces Gag precursor protein and the gag-pol fusion protein by frame shifting in a 20:1 ratio (Wilson, W et al., 1988, Cell, 55:1159-1169). The deletion of a frame shift site in hGag-Pol Δ FS Δ Pr results in production of only the Gag-Pol fusion protein. Expression of Gag-Pol proteins alone in human cells is not adequate to form releasable viral particles because HIV-1 viral assembly requires Gag precursor proteins (Park, J and CD Morrow, 1992, J Virol, 66:6304-6313; Smith, AJ et al., 1993, J Virol, 67:2266-2275). The ability of hGag-Pol Δ FS Δ Pr to elicit strong Gag and Pol specific CTL responses in mice may be explained by high level expression of Gag-Pol fusion protein and its retention on the inside of cells, which could create a new condition not existing during normal viral replication and could provide sufficient proteins for antigen presentation. Moreover, the mutation in viral protease prevents viral protein from intracellular activation and reduced cellular toxicity. Overexpression of this polyprotein is also likely to affect its intracellular localization/transport and is envisioned as improving antigen presentation.

The Pol gene of HIV-1 is the precursor protein for viral protease, reverse transcriptase and integrase, which are crucial to viral replication (Kohl, NE et al., 1988, Proc. Natl. Acad. Sci. USA, 85:4686-4690.). Retroviral extracellular maturation, resulting from self-activation of protease after release produces mature RT and IN which have important functions in reverse transcription and integration respectively, for HIV-1, HIV-2, and SIV replication. The catalytic cores of these

enzymes have relatively conserved domains in order to preserve their functions and thus are envisioned as inducing cross-clade CTL responses. As early as 1988, CTLs specific for HIV-1 RT were found in blood samples from HIV-1 infected individuals (Hosmalin, A et al., 1990, Proc. Natl. Acad. Sci. USA, 87:2344-2348; Walker, BD et al., 1988, Science, 240:64-66.). Relatively strong Gag-specific CTL responses have been shown in numerous non-human primate and human studies, using DNA vaccines or a live recombinant vector containing viral Gag-Pol constructs (Evans, TG, 1999, J Infect Dis, 180:290-298; Ferrari, G, 1997, Blood, 90:2406-2416; Gorse, GJ et al., 1999, Vaccine, 18:835-849; Seth, A, 1998, Proc. Natl. Acad. Sci. USA, 95:10112-10116; Seth, A et al., 2000, J Virol, 74:2502-2509), but fewer Pol-specific CTL responses have been reported. The detection of significant CTL responses specific to Pol in our disclosure may be attributed in part to establishment of stable Pol expressing cell lines, in which codon alteration and inactivation of FS and PR in the Pol gene allow high level expression of the Pol protein without cellular toxicity. Though it remains possible that the hGag-Pol, or a combination of hGag and hPol, may exert similar effects with appropriate adjuvants or with different prime-boost regimens, the Rev-independent Gag-Pol fusion protein stimulates HIV-1 Gag and Pol specific CTL responses in mice and is envisioned as proving useful in an AIDS vaccine.

PLASMID DESCRIPTIONS

Env Plasmids

VRC2100

pVR1012x/s R5gp139-Nef (delta) MHC (delta) CD4/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. The envelope protein gene from pX4gp160/h (Nabel lab #1272) was ligated in frame with the mutant Nef gene from pNefDMHCDCD4/h (Nabel lab #1278) to produce

pX4gp139-NefDMHCDCD4/h. The envelope-Nef fusion protein expressed from pX4gp139-NefDMHCDCD4/h contains the first 668 amino acids from the HIV envelope glycoprotein (gp139) fused to the entire mutant Nef protein. The truncated envelope polyprotein (gp139) contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain and regions important for oligomer formation. The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit both MHC class I and CD4 expression, point mutations were introduced into the Nef gene from pNef/h (Nabel lab #1275). The resulting amino acids substitutions in pNefDMHCDCD4/h are: P69A, P72A, P75A, P78A, D174A and D175A. X4gp139-NefDMHCDCD4/h is expressed from the pVR1012x/s (Nabel lab #1267) vector backbone.

15 VRC2200

pVR1012x/s R5gp157-Nef (delta) MHC (delta) CD4/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (Nabel lab number 1272) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The envelope-Nef fusion protein expressed from pR5gp157-Nef/h contains the first 820 amino acids from the HIV envelope glycoprotein (gp157) fused to the entire mutant Nef protein. The gene for gp157 was ligated in frame with the full-length mutant Nef gene from pNefDMHCDCD4/h (Nabel lab #1278) to produce pR5gp157-NefDMHCDCD4/h. The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit both MHC class I and CD4 expression, point mutations

were introduced into the Nef gene from pNef/h (Nabel lab #1275). The resulting amino acids substitutions in pNefDMHCDCD4/h are: P69A, P72A, P75A, P78A, D174A and D175A. R5gp157-NefDMHCDCD4/h is expressed from the pVR1012x/s (Nabel lab #1267) vector backbone.

5

VRC2300

pVR1012x/s X4 gp139-Nef deltaMHC deltaCD4/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. The envelope protein gene from pX4gp160/h (Nabel lab #1272) was ligated in frame with the mutant Nef gene from pNefDMHC/h (Nabel lab #1276) to produce pX4gp139-NefDMHC/h. The envelope-Nef fusion protein expressed from pX4gp139-NefDMHC/h contains the first 668 amino acids from the HIV envelope glycoprotein (gp139) fused to the entire mutant Nef protein. The truncated envelope polyprotein (gp139) contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain and regions important for oligomer formation. The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit MHC class I expression, point mutations were introduced into the Nef gene from pNef/h (Nabel lab #1275). The resulting amino acids substitutions in pNefDMHC/h are: P69A, P72A, P75A, and P78A. X4gp139-NefDMHC/h is expressed from the pVR1012x/s (Nabel lab #1267) vector backbone

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VRC2302

pVR1012x/s X4gp130-Nef/h

For the X4gp130/h (VRC2703) portion, the protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons

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optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. The full-length X4-tropic version of the envelope protein from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 602. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. Regions important for oligomer formation may be partially functional. This X4gp130/h (VRC2703) gene was fused to Nef gene. The Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create the viral Nef gene (pVR1012-Nef)(Nabel Lab #1093. The nucleotide sequence is homologous to the viral gene, and the protein encoded is the same. The expression vector backbone is pVR1012x/s (VRC2000).

VRC2400

pVR1012x/s X4gp157-NefDMHCDCD4/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. The envelope-Nef fusion protein expressed from pX4gp157-NefDMHCDCD4/h contains the first 820 amino acids from the HIV envelope glycoprotein (gp157) fused to the entire mutant Nef protein. The truncated envelope polyprotein (gp157) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The gene for gp157 was ligated in frame with the full-length mutant Nef gene from pNefDMHCDCD4/h (VRC3600) to produce pX4gp157-NefDMHCDCD4/h. The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit both

MHC class I and CD4 expression, point mutations were introduced into the Nef gene from pNef/h (VRC3500). The resulting amino acids substitutions in pNefDMHCDCD4/h are: P69A, P72A, P75A, P78A, D174A and D175A. X4gp160-NefDMHCDCD4/h is expressed from the pVR1012x/s (VRC2000) vector backbone.

VRC2700

pVR1012x/s X4gp140/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. The full-length X4-tropic version of the envelope protein from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC2701

pVR1012x/s X4gp140(del F/CL del H IS)/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. The full-length X4-tropic version of the envelope protein from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking

the transmembrane domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC2702

pVR1012x/s X4gp128(del F/CL)/h

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The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. The full-length X4-tropic version of the envelope protein from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 592. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC2706

pVR1012x/s X4gp145/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. The full-length X4-tropic version of the envelope protein from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 704. The truncated envelope polyprotein contains the entire

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SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC2707

pVR1012x/s X4gp145(del F/CL del H IS)/h

10 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. The full-length X4-tropic version
15 of the envelope protein from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 704. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for
20 oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC2800

pVR1012x/s R5gp140/h

25 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an
30 R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were
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replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC2801

pVR1012x/s R5gp140(del F/CL del H IS)/h

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an
10 R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was
15 terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between
20 Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC2804

25 pVR1012x/s R5gp145/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
30 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with
35 the corresponding region from the BaL strain of HIV-1 (GeneBank accession

number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC2805

pVR1012x/s R5gp145(del F/CL del H IS)/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2810

pVR1012 x/s R5gp140delC1(delCFI)/h

5 Constant domain 1 was deleted from gp140delCFI from amino acid 33-127 and was replaced with a NheI site.

VRC 2811

pVR1012 x/s R5gp140delC2 (delCFI)/h

10 Constant domain 2 was deleted from gp140delCFI from amino acid 199-293, and was replaced with an NheI site

VRC2812

pVR 1012 x/s R5gp140delC3 (delCFI)/h

15 Constant domain 3 was deleted from gp140delCFI from amino acid 333-380, and was replaced with an NheI site.

VRC2813

20 pVR1012 x/s R5gp140delC4 (delCFI)/h

Constant domain 4 was deleted from gp140delCFI from amino acids 419-458, and was replaced with an NheI site.

VRC2814

pVR1012 x/s R5gp140delC5 (delCFI)/h

25 Constant domain 5 was deleted from gp140delCFI from amino acid 472 -498 and was replaced with an NheI site.

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VRC 2820

pVR1012x/s R5gp140(dCFI)/dV1

35 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the

gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1 loop (a.a.129 to 154) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2821

pVR1012x/s R5gp140(dCFI)/dV2

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V2 loop (a.a.160 to 193) and transmembrane domain.

Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2822

pVR1012x/s R5gp140(dCFI)/dV3

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V3 loop (a.a.299 to 327) and transmembrane domain. Heptad (H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2823

pVR1012x/s R5gp140(dCFI)/dV4

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The

nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polypeptide amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polypeptide contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V4 loop (a.a.386 to 413) and transmembrane domain. Heptad (H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2824

pVR1012x/s R5gp140(dCFI)/dV12

The protein sequence of the envelope polypeptide (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polypeptide amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polypeptide contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1, V2 loops (a.a.129 to 154, and a.a.160 to 193) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required

for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2825

pVR1012x/s R5gp140(dCFI)/dV13

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1, V3 loops (a.a.129 to 154, and a.a.299 to 327) and transmembrane domain. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2826

pVR1012x/s R5gp140(dCFI)/dV14

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the

gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1, V4 loops (a.a.129 to 154, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

20 VRC 2827

pVR1012x/s R5gp140(dCFI)/dV23

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion

domain, but lacking the V2, V3 loops (a.a.160 to 193, and a.a.299 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2828

10 pVR1012x/s R5gp140(dCFI)/dV24

The protein sequence of the envelope polypeptide (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polypeptide amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polypeptide contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V2, V4 loops (a.a.160 to 193, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2829

pVR1012x/s R5gp140(dCFI)/dV34

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an

10 R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was

15 terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V3, V4 loops (a.a.299 to 327, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids

20 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2830

pVR1012x/s R5gp140(dCFI)/dV123

30 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an

35 R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were

replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1,V2,V3 loops (a.a. 129 to 154, a.a.160 to 193,and a.a.299 to 327) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2831

pVR1012x/s R5gp140(dCFD)/dV124

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1,V2,V4 loops (a.a. 129 to 154, a.a.160 to 193,and a.a.386 to 413) and transmembrane domain. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for

oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2832

5 pVR1012x/s R5gp140(dCFI)/dV134

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
10 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were
15 replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion
20 domain, but lacking the V1,V3,V4 loops (a.a. 129 to 154, a.a.299 to 327, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for
25 oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2833

30 pVR1012x/s R5gp140(dCFI)/dV234

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
35 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L,

N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank
5 accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V2,V3,V4 loops (a.a. 160 to 193, a.a.299 to 327, and a.a.386
10 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is
15 pVR1012x/s (VRC2000).

VRC 2834

pVR1012x/s R5gp140(dCFI)/dV1234

20 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L,
25 N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length
30 R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1,V2,V3,V4 loops (a.a. 129 to 154, a.a. 160 to 193, a.a.299 to 327, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad
35 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage

(F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC 2835

pAdApt R5gp140(dCFI)/dV1

10 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an
15 R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was
20 terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1 loop (a.a.129 to 154) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been
25 deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

VRC 2836

30 pAdApt R5gp140(dCFI)/dV2

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
35 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the

protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V2 loop (a.a.160 to 193) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is AdApt.

VRC 2837

pAdApt R5gp140(dCFI)/dV3

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V3 loop (a.a.299 to 327) and transmembrane domain. Heptad (H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been

deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

5 **VRC 2838**

pAdApt R5gp140(dCFI)/dV4

10 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1
15 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein
20 contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V4 loop (a.a.386 to 413) and transmembrane domain. Heptad (H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620,
25 have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

VRC 2839

pAdApt R5gp140(dCFI)/dV12

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The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the
35 protein encoded is the same with the following amino acid substitutions: F53L,

N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polypeptide amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank
5 accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polypeptide contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1, V2 loops (a.a.129 to 154, and a.a.160 to 193) and
10 transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

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VRC 2840**pAdApt R5gp140(dCFI)/dV13**

The protein sequence of the envelope polypeptide (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the
20 gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polypeptide amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was
25 terminated after the codon for amino acid 680. The truncated envelope polypeptide contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1, V3 loops (a.a.129 to 154, and a.a.299 to 327) and transmembrane domain. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino
30 acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2,
35

from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

VRC 2841

5 pAdApt R5gp140(dCFI)/dV14

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
10 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were
15 replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion
20 domain, but lacking the V1, V4 loops (a.a.129 to 154, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation
25 may be partially functional. The expression vector backbone is pAdApt.

VRC 2842

pAdApt R5gp140(dCFI)/dV23

30 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L,
35 N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an

R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length
5 R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V2, V3 loops (a.a.160 to 193, and a.a.299 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required
10 for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

15 **VRC 2843**

pAdApt R5gp140(dCFI)/dV24

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the
20 gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1
25 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein
30 contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V2, V4 loops (a.a.160 to 193, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from

amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

VRC 2844

5 pAdApt R5gp140(dCFI)/dV34

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
10 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were
15 replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion
20 domain, but lacking the V3, V4 loops (a.a.299 to 327, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation
25 may be partially functional. The expression vector backbone is pAdApt.

VRC 2845

pAdApt R5gp140(dCFI)/dV123

30 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L,
35 N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an

R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length
5 R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1,V2,V3 loops (a.a. 129 to 154, a.a.160 to 193,and a.a.299 to 327) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS)
10 are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt

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VRC 2846

pAdApt R5gp140(dCFI)/dV124

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the
20 gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an
25 R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was
30 terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1,V2,V4 loops (a.a. 129 to 154, a.a.160 to 193,and a.a.386 to 413) and transmembrane domain. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains,
35 from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad

(H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

5 **VRC 2847**

pAdApt R5gp140(dCFI)/dV134

10 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1
15 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein
20 contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1,V3,V4 loops (a.a. 129 to 154, a.a.299 to 327, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1
25 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

30 **VRC 2848**

pAdApt R5gp140(dCFI)/dV234

35 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the

protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V2,V3,V4 loops (a.a. 160 to 193, a.a.299 to 327, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

VRC 2849

pAdApt R5gp140(dCFI)/dV1234

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 680. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the V1,V2,V3,V4 loops (a.a. 129 to 154, a.a. 160 to 193, a.a.299 to 327, and a.a.386 to 413) and transmembrane domain. Heptad(H) 1, Heptad

2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pAdApt.

VRC2850

pVR1012 x/s R5gp145delC1 (delCFI)/h

Constant domain 1 was deleted from gp145delCFI from amino acid 33-127 and was replaced with an Nhe I site.

VRC2851

pVR1012 x/s R5gp145delC2 (delCFI)/h

Constant domain 2 was deleted from gp145dCFI from amino acid 199-293 and was replaced with an Nhe I site.

VRC2852

pVR1012 x/s R5gp145delC3 (delCFI)/h

Constant domain 3 was deleted from gp145delCFI from amino acids 333-380 and was replaced with an Nhe I site.

VRC2853

pVR1012 x/s R5gp145delC4 (delCFI)/h

Constant domain 4 was deleted from gp145delCFI from amino acid 419-458 and was replaced with an Nhe I site.

VRC2854

pVR1012 x/s R5gp145delC5 (delCFI)/h

Constant domain 5 was deleted from gp145delCFI from amino acid 472-498 and was replaced with an Nhe I site.

VRC 2860

pVR1012x/s R5gp145(dCFI)/h/dV1

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The V1 loop (a.a. 129-154) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2861

pVR1012x/s R5gp145(dCFI)/h/dV2

30 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with

the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The V2 loop (a.a. 160-193) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2862

pVR1012x/s R5gp145(dCFD)/h/dV3

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The V3 loop (a.a. 299-327) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2863

pVR1012x/s R5gp145(dCFI)/h/dV4

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version
10 of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated
15 after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V4 loop (a.a. 386-413) and Fusion and Cleavage (F/CL) domains (a.a. 503-536)
20 have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2864

25 pVR1012x/s R5gp145(dCFI)/h/dV12

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
30 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with
35 the corresponding region from the BaL strain of HIV-1 (GeneBank accession

number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The V1, V2 loops (a.a. 129-154 and 160-193) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2865

pVR1012x/s R5gp145(dCFI)/h/dV13

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V1, V3 loops (a.a. 129-154 and 299-327) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2866

pVR1012x/s R5gp145(dCFI)/h/dV14

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version
10 of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated
15 after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V1, V4 loops (a.a. 129-154 and 386-413) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad
20 (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2867

25 pVR1012x/s R5gp145(dCFI)/h/dV23

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
30 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with
35 the corresponding region from the BaL strain of HIV-1 (GeneBank accession

number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V2, V3 loops (a.a. 160-193 and 299-327) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2868

pVR1012x/s R5gp145(dCFI)/h/dV24

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V2, V4 loops (a.a. 160-193 and 386-413) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2869

pVR1012x/s R5gp145(dCFD)/h/dV34

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version
10 of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated
15 after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V3, V4 loops (a.a. 299-327 and 386-413) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad
20 (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2870

25 pVR1012x/s R5gp145(dCFI)/h/dV134

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
30 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with
35 the corresponding region from the BaL strain of HIV-1 (GeneBank accession

number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V1, V3, V4 loops (a.a. 129-154, 299-327 and 386-413) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2871

pVR1012x/s R5gp145(dCF1)/h/dV234

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The V2, V3, V4 loops (a.a. 160-193, 299-327 and 386-413) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2872

pVR1012x/s R5gp145(dCFI)dv123/h

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version
10 of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated
15 after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V1, V2, V4 loops (a.a. 129-154, 160-193 and 386-413) and Fusion and
20 Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2873

25 pVR1012x/s R5gp145(dCFI)/h/dV124

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The
30 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with
35 the corresponding region from the BaL strain of HIV-1 (GeneBank accession

number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V1, V2, V4 loops (a.a. 129-154, 160-193 and 386-413) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 2874

pVR1012x/s R5gp145(dCFI)/h/dV1234

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, and P470L. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 704. The truncated envelope polyprotein (gp145) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad (H) 1, Heptad 2 and their Interspace (IS) are required for oligomerization. The V1, V2, V3, V4 loops (a.a. 129-154, 160-193, 299-327 and 386-413) and Fusion and Cleavage (F/CL) domains (a.a. 503-536) have been deleted. Also, the Interspace (IS) between Heptad (H) 1 and 2 (a.a.593-620) have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC2900

pVR1012x/s R5gp150/h

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The full-length R5-tropic version of the envelope protein gene from pR5gp160/h (VRC3000) was terminated after the codon for amino acid 752. The truncated envelope polyprotein (gp150) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. The expression vector backbone is pVR1012x/s (VRC2000).

VRC3000

pVR1012x/s R5gp160/h

25 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). Full length SU and TM proteins are expressed from the pVR1012x/s (VRC2000) vector backbone.

VRC3200

pVR1012x/s X4gp150/h

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. The full-length X4-tropic version of the envelope protein gene from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 752. The truncated envelope polyprotein (gp150) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC3201

pVR1012x/s X4gp150(del F/CL del H IS)/h

20 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. The full-length X4-tropic version of the envelope protein gene from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 752. The truncated envelope polyprotein (gp150) contains the entire SU protein and a portion of the TM protein including the fusion domain, the transmembrane domain, and regions important for oligomer formation. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC3202

pVR1012x/s X4gp150 Δgly/h.

5 Eukaryotic vector with humanized codons expressing the HIV envelope glycoprotein gp150 from HXB2, X4 tropic mutated in the Glycosylation sites. VRC3202 pVR1012x/s X4gp150Dgly/h The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4- tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows
 10 little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To disrupt potential glycosylation sites in the HIV-1 envelope proteins, point mutations were introduced into the full-length X4-tropic version of the envelope protein gene from pX4gp160/h (VRC3300). The resulting
 15 amino acids substitutions in X4gp160Dgly/h are: N88D, N156D, N160D, N197E, N230D, N234D, N241D, N276D, L288V, N289D, S291T, N295D, N332D, N339D, N356D, N386D, and N448D. The full-length X4-tropic version of the envelope protein gene from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 752. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for
 20 oligomerization. Full length SU and TM proteins are expressed from the pVR1012x/s (VRC2000) vector backbone.

VRC3203

pVR1012x/s X4gp150 AB Δgly/h

25 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The AB designation means the amino acids from 1-307 (XbaI to EcoRI). The nucleotide
 30 sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To disrupt potential glycosylation sites in the HIV-1 envelope proteins, point mutations were introduced into the full-length X4-tropic version of the envelope protein gene from pX4gp160/h

(VRC3300). The resulting amino acids substitutions in X4gp160Dgly/h are: N88D, N156D, N160D, N197E, N230D, N234D, N241D, N276D, L288V, N289D, S291T, and N295D. The full-length X4-tropic version of the envelope protein gene from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 752. 5 Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. Full length SU and TM proteins are expressed from the pVR1012x/s (VRC2000) vector backbone.

VRC3300

10 pVR1012x/s X4gp160/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The 15 nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. Full length SU and TM proteins are expressed from the pVR1012x/s (VRC2000) vector backbone.

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VRC3301

pVR1012x/s X4gp160(del F/CL del H IS)/h

The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, 25 GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. Full length SU 30 and TM proteins are Expressed. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC3400

pVR1012x/s X4gp160Δgly/h

5 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To disrupt potential glycosylation sites in the HIV-1 envelope proteins, point mutations were introduced into the full-length X4-tropic version of the envelope protein gene from pX4gp160/h (VRC3300). The resulting amino acids substitutions in X4gp160Dgly/h are: N88D, N156D, N160D, N197E, N230D, N234D, N241D, N276D, L288V, N289D, S291T, N295D, N332D, N339D, N356D, N386D, and N448D. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. Full length SU and TM proteins are expressed from the pVR1012x/s (VRC2000) vector backbone.

VRC3401

pVR1012x/s X4gp160AB mut Δgly/h

20 The protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The AB designation means the amino acids from 1-307 (XbaI to EcoR1). The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To disrupt potential glycosylation sites in the HIV-1 envelope proteins, point mutations were introduced into the full-length X4-tropic version of the envelope protein gene from pX4gp160/h (VRC3300). The resulting amino acids substitutions in X4gp160Dgly/h are: N88D, N156D, N160D, N197E, N230D, N234D, N241D, N276D, L288V, N289D, S291T, and N295D. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. Full length SU and TM proteins are expressed from the pVR1012x/s (VRC2000) vector backbone.

VRC3500

pVR1012x/s Nef/h

5 The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. The nucleotide sequence Nef/h shows little homology to the viral gene, but the protein encoded is the same. Nef/h is expressed from the pVR1012x/s (VRC2000) vector backbone.

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VRC3600

pVR1012x/s NefDMHCD4/h

15 The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit both MHC class I and CD4 expression point mutations were introduced into the Nef gene from pNef/h (VRC3500). The resulting amino acids substitutions in pNefDMHCD4/h are: P69A, P72A, P75A, P78A, D174A and D175A. pNefDMHCD4/h is expressed from the pVR1012x/s (VRC2000) vector backbone.

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VRC3700

pVR1012x/s NefDCD4/h

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The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit CD4 expression, point mutations were introduced into the Nef gene from pNef/h (VRC3500). The resulting amino acids substitutions in pNefDCD4/h are: D174A and D175A. pNefDCD4/h is expressed from the pVR1012x/s (VRC2000) vector backbone. VRC3700 pVR1012x/s NefDCD4/h The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit CD4 expression,

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point mutations were introduced into the Nef gene from pNef/h (VRC3500). The resulting amino acids substitutions in pNefDCD/h are: D174A and D175A. pNefDCD4/h is expressed from the pVR1012x/s (VRC2000) vector backbone.

5 **VRC3800**

pVR1012x/s NefDMHC/h

10 The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit MHC class I expression, point mutations were introduced into the Nef gene from pNef/h (VRC3500). The resulting amino acids substitutions in pNefDMHC/h are: P69A, P72A, P75A, and P78A. pNefDMHC/h is expressed from the pVR1012x/s (VRC2000) vector backbone.

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VRC5200

pVR1012x/s 89.6Pgp128(del F/CL)/h

20 The protein sequence of the envelope polyprotein (gp160) from 89.6P (Dual-tropic, GenBank accession number u89134/LOCUS:SIU89134) was used to create a synthetic version of the gene (89.6Pgp160/h) using codons optimized for expression in human cells. The nucleotide sequence 89.6Pgp160/h shows little homology to the 89.6P gene, but the protein encoded is the same. The full-length 89.6P, dual-tropic version of the envelope protein gene from 89.6P gp160/h (VRC3000) was terminated after the codon for amino acid 596. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. The Fusion and Cleavage (F/CL) domains, from amino acids 508-541, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC5201

pVR1012x/s 89.6Pgp140(del F/CL del H IS)/h

35 The protein sequence of the envelope polyprotein (gp160) from 89.6P (dual-tropic, GenBank accession number u89134/locus SIU89134) was used to create a synthetic

version of the gene (Dualtropic gp160/h) using codons optimized for expression in human cells. The nucleotide sequence dualtropic gp160/h shows little homology to the 89.6P gene, but the protein encoded is the same. The full-length 89.6P, dual-tropic version of the envelope protein gene was terminated after the codon for amino acid 683. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 508-541, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 597-625, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5202

pVR1012x/s 89.6Pgp145(del F/CL del H IS/h

The protein sequence of the envelope polyprotein (gp160) from 89.6P (dual-tropic, GenBank accession number u89134/locus SIU89134) was used to create a synthetic version of the gene (Dualtropic gp160/h) using codons optimized for expression in human cells. The nucleotide sequence dualtropic gp160/h shows little homology to the 89.6P gene, but the protein encoded is the same. The full-length 89.6P, dual-tropic version of the envelope protein gene was terminated after the codon for amino acid 709. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 508-541, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 597-625, have been deleted. Regions important for oligomer formation may be partially functional. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5203

pVR1012x/s 89.6Pgp160/h

5 The protein sequence of the envelope polyprotein (gp160) from 89.6P (Dual-tropic, GenBank accession number U89134/LOCUS: SIU89134) was used to create a synthetic version of the gene (dual tropic gp160/h) using codons optimized for expression in human cells. The nucleotide sequence 89.6P gp160/h shows little homology to the 89.6P gene, but the protein encoded is the same. Full length SU and TM proteins are expressed from the pVR1012x/s (VRC2000) vector backbone

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VRC5300

pVR1012x/s R5(clade C)gp140(del F/CL del H IS)/h

15 The protein sequence of the envelope polyprotein (gp160) from 92br025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (R5gp160/h) using codons optimized for expression in human cells. The nucleotide sequence R5gp160/h shows little homology to the 92br025 gene, but the protein encoded is the same. The full-length R5-tropic version of the envelope protein was synthesized by Operon under the name: kongene. The XbaI (18nt up-stream from ATG) to BglII (1376nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BglII sites of VRC2701 pVR1012x/s X4gp140(del F/CL del H IS)/h backbone. Therefore, the gene is R5 (clade C) gp160/h up to the BglII site (1376nt from ATG) and the rest of the gene after BglII site is VRC2701 pVR1012x/s X4gp140(del F/CL del H IS)/h. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000). The full-length X4-tropic version of the envelope protein from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 680.

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VRC5301

pVR1012x/s R5(clade C)gp145(del F/CL del H IS)/h

5 The protein sequence of the envelope polyprotein (gp160) from 92br025 (R5-tropic, GenBank accessionnumber U52953) was used to create a synthetic version of the gene (R5gp160/h) using codons optimized for expression in human cells. The nucleotide sequence R5gp160/h shows little homology to the 92br025 gene, but the protein encoded is the same. The full-length R5-tropic version of the envelope protein was synthesized by Operon under the name: kongene. The XbaI (18nt up-
10 stream from ATG) to BglII (1376nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BglII sites of VRC2701 pVR1012x/s X4gp140(del F/CL del H IS)/h backbone. Therefore, the gene is R5 (clade C) gp160/h up to the BglII site (1376nt from ATG) and the rest of the gene after BglII site is VRC2707 pVR1012x/s
15 X4gp145(del F/CL del H IS)/h. The truncated envelope polyprotein contains the entire SU protein and a portion of the TM protein including the fusion domain, but lacking the transmembrane domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids
20 503-536, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 593-620, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000). The full-length X4-tropic version of the envelope protein from pX4gp160/h (VRC3300) was terminated after the codon for amino acid 704.

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VRC5303

pVR1012x/s R5gp145CladeC(Brazil) delCFI/h

Authentic Clade C unlike 5301 which is a hybrid between C and B.

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VRC 5304

pVR1012x/s R5(clade A)gp140(del F/CL del H IS)/h

35 The protein sequence of the envelope polyprotein (gp160) from 92rw020 (R5-tropic, GenBank accessionnumber U51283) was used to create a synthetic version of the

gene (Clade-A gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the 92rw020 gene, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1837 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains the entire SU protein and the TM domain, but lacking the the Fusion domain and Cytoplasmic domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 486-519, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 576-604, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5305

pVR1012x/s R5(clade A)gp145(del F/CL del H IS)/h

The protein sequence of the envelope polyprotein (gp160) from 92rw020 (R5-tropic, GenBank accessionnumber U51283) was used to create a synthetic version of the gene (Clade-A gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the 92rw020 gene, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1912 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains the entire SU protein and the TM domain, but lacking the the Fusion domain and Cytoplasmic domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 486-519, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 576-604, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5306

pVR1012x/s R5(clade E)gp140(del F/CL del H IS)/h

5 The protein sequence of the envelope polyprotein (gp140delCFI) from 93th966.8 (R5-tropic, GenBank accessionnumber U08456) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 93th966.8, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1856 nt down-stream from ATG) fragment which contains
10 polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains the entire SU protein and the TM domain, but lacking the the Fusion domain and Cytoplasmic domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for
15 oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 497-530, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 588-613, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5307

pVR1012x/s R5(clade E)gp145(del F/CL del H IS)/h

25 The protein sequence of the envelope polyprotein (gp145delCFI) from 93th966.8 (R5-tropic, GenBank accessionnumber U08456) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 93th966.8, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1928 nt down-stream from ATG) fragment which contains
30 polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains the entire SU protein and the TM domain, but lacking the the Fusion domain and Cytoplasmic domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for
35 oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 497-530, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from

amino acids 588-613, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5308

5 pVR1012x/s R5(clade C South African)gp140(del F/CL del H IS)/h

The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accessionnumber AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression
10 in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamH1 (1833 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamH1 sites of pVR1012x/s backbone. The truncated envelope polyprotein
15 contains the entire SU protein and the TM domain, but lacking the the Fusion domain and Cytoplasmic domain. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 487-520, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from
20 amino acids 577-605, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5309

25 pVR1012x/s R5(clade C South African)gp145(del F/CL del H IS)/h

The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accessionnumber AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression
30 in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamH1 (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamH1 sites of pVR1012x/s backbone. The truncated envelope polyprotein
35 contains the entire SU protein and the TM domain, but lacking the the Fusion domain and Cytoplasmic domain. Regions important for oligomer formation may be

partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The Fusion and Cleavage (F/CL) domains, from amino acids 487-520, have been deleted. The Interspace (IS) between Heptad (H) 1 and 2, from amino acids 577-605, have been deleted. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5350

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV1

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1 loop(a.a.133-148) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5351

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV12

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into

the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polypeptide contains deletion in the V1,V2 loops (a.a.133-191) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

10 **VRC 5352**

pVR1012(x/s)-gp140(dCFI)(Brazil C)/dV123

The protein sequence of the envelope polypeptide (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polypeptide contains deletion in the V1,V2,V3 loops (a.a.130-191, 330-358) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

25 **VRC 5353**

30 pVR1012(x/s)-gp140(dCFI)(Brazil C)/dV1234

The protein sequence of the envelope polypeptide (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little

homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1,V2,V3,V4 loops (a.a.130-191, 330-358, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5354

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV124

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1,V2,V4 loops (a.a. 130-191, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5355

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV13

5 The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment
10 which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1,V3 loops (a.a.133-148, 330-358) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain,
15 and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5356

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV134

25 The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment
30 which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1,V3,V4 loops (a.a.130-148, 330-358, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions
35 important for oligomer formation may be partially functional. Heptad(H) 1, Heptad

2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5357

5 pVR1012(x/s)-gp140(dCFI)(Brazil C)/dV14

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little
10 homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope
15 polyprotein contains deletion in the V1,V4 loops (a.a. 130-148, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer
20 formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5358

25 pVR1012(x/s)-gp140(dCFI)(Brazil C)/dV2

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little
30 homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope
polyprotein contains deletion in the V2 loop(a.a.154-191) of the SU protein. It also
35 lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS)

between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5359

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV23

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V2,V3 loops (a.a. 154-191, 330-358) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5360

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV234

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into

the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V2,V3,V4 loops (a.a.154-191, 330-358, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612),
5 transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

10 **VRC 5361**

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV24

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version
15 of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into
20 the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V2,V4 loops (a.a. 154-191, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer
25 formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5362

30 pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV3

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in
35 human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little

homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V3 loop(a.a.330-358) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5363

pVR1012(x/s)-gp140(dCFI)(Brazil C)/dV34

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V3,V4 loops (a.a.330-358, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5364

pVRC1012(x/s)-gp140(dCFI)(Brazil C)/dV4

5 The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp140(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment
10 which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V4 loop(a.a.384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), transmembrane domain, and
15 cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5365

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV1

25 The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment
30 which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1 loop(a.a.133-148) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional.

Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5366

5 PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV12

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little
10 homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope
15 polyprotein contains deletion in the V1,V2 loops (a.a.133-191) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The
20 expression vector backbone is pVR1012x/s (VRC2000).

VRC 5367

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV123

25 The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little
30 homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1, V2,V3 loops (a.a. 133-191,330-358) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529),
35 the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic

domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

5 **VRC 5368**

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV1234

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little
10 homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into
15 the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1,V2, V3,V4 loops (a.a. 133-191,330-358,384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and
20 Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5369

25 PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV124

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little
30 homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope
35 polyprotein contains deletion in the V1, V2,V4 loops (a.a. 133-148,154-191,384-

408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5370

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV13

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The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1,V3 loops (a.a.133-148, 330-358) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC 5371

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV134

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into

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the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1, V3, V4 loops (a.a. 133-148, 330-358, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains (a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2 (a.a. 586-612), and
5 Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

10 **VRC 5372**

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV14

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version
15 of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into
20 the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V1, V4 loops (a.a. 133-148, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains (a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2 (a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially
25 functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5373

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV2

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The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little
35 homology to the gene 92BR025, but the protein encoded is the same. The XbaI

(18nt up-stream from ATG) to BamH1 (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamH1 sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V2 loop (a.a.154-191) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC 5374

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV23

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The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamH1 (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamH1 sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V2,V3 loops (a.a.154-191,330-358) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC 5375

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV234

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The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little

homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V2, V3,V4 loops (a.a. 154-191,330-358,384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5376

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV24

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V2,V4 loops (a.a.154-191, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5377

PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV3

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version

of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment
5 which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V3 loop (a.a.330-358) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM
10 protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5378

15 PVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV34

The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(delCFI) shows little
20 homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1910 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope
25 polyprotein contains deletion in the V3,V4 loops (a.a.330-358, 384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM protein. Regions important for oligomer formation may be partially
30 functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5379

pVRC1012(x/s)-gp145(dCFI)(Brazil C)/dV4

5 The protein sequence of the envelope polyprotein (gp160) from 92BR025 (R5-tropic, GenBank accession number U52953) was used to create a synthetic version of the gene (Brazil-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence of Brazil-C gp145(dCFI) shows little homology to the gene 92BR025, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamH1 (1910 nt down-stream from ATG) fragment
10 which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamH1 sites of pVR1012x/s backbone. The truncated envelope polyprotein contains deletion in the V4 loop (a.a.384-408) of the SU protein. It also lacks the Fusion and Cleavage (F/CL) domains(a.a. 496-529), the Interspace (IS) between Heptad (H) 1 and 2(a.a. 586-612), and Cytoplasmic domain in the TM.
15 protein. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5500

20 pVR1012x/s R5(SA-C)gp140(dCFI) dV1/h

The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression
25 in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamH1 (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamH1 sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks
30 the V1 loop (a.a.136-150), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC5501

pVR1012x/s R5(SA-C)gp140(dCFI) dV12/h

5 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains
10 polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V2 loops (a.a.136-194), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer
15 formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC 5502

20 pVR1012x/s R5(SA-C)gp140(dCFI) dV123/h

The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression
25 in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks
30 the V1, V2, V3 loops (a.a. 136-194, 297-325), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is
35 pVR1012x/s (VRC2000).

VRC5503

pVR1012x/s R5(SA-C)gp140(dCFI)dV1234/h

5 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V2, V3, V4 loops (a.a.136-194,297-325, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5504

pVR1012x/s R5(SA-C)gp140(dCFI)dV124/h

25 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V2, V4 loops (a.a. 136-194, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their

Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5505

5 pVR1012x/s R5(SA-C)gp140(dCFI)dV13/h

The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression
 10 in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks
 15 the V1, V3 loops (a.a.136-150, 297-325), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is
 20 pVR1012x/s (VRC2000).

VRC5506

pVR1012x/s R5(SA-C)gp140(dCFI)dV134/h

25 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream
 30 from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V3, V4 loops (a.a. 136-150, 297-325, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2
 35 (a.a.577-605), the transmembrane domain and the intracellular region. Regions

important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

5 **VRC5507**

pVR1012x/s R5(SA-C)gp140(dCFI)dV14/h

10 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V4 loops (a.a.136-150, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

15 **VRC5508**

pVR1012x/s R5(SA-C)gp140(dCFI)dV2/h

25 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V2 loop (a.a.156-194), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane

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domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

5 **VRC5509**

pVR1012x/s R5(SA-C)gp140(dCFI)dV23/h

10 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to
15 BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V2, V3 loops (a.a.156-194, 297-325), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their
20 Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5510

pVR1012x/s R5(SA-C)gp140(dCFI)dV234/h

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30 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V2, V3, V4 loops (a.a. 156-194, 297-325, 385-399), the Fusion and Cleavage
35 (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2

(a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC5511

pVR1012x/s R5(SA-C)gp140(dCFI)dV24/h

10 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains
15 polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V2, V4 loops (a.a.156-194, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer
20 formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5512

25 pVR1012x/s R5(SA-C)gp140(dCFI)dV3/h

The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression
30 in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks
35 the V3 loop (a.a.297-325), the Fusion and Cleavage (F/CL) domains (a.a. 487-520),

the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC5513

pVR1012x/s R5(SA-C)gp140(dCFI)dV34/h

10 The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains
15 polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V3, V4 loops (a.a. 297-325, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer
20 formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5514

25 pVR1012x/s R5(SA-C)gp140(dCFI)dV4/h

The protein sequence of the envelope polyprotein (gp140delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp140delCFI) using codons optimized for expression
30 in human cells. The nucleotide sequence R5gp140delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks
35 the V4 loop (a.a 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520),

the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), the transmembrane domain and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC5515

pVR1012x/s R5(SA-C)gp145(dCFI)dV1/h

10 The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains
15 polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1 loop (a.a.136-150), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially functional.
20 Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5516

pVR1012x/s R5(SA-C)gp145(dCFI)dV12/h

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The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to
30 the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1,V2 loops (a.a.136-194), the Fusion and Cleavage (F/CL) domains (a.a. 487-
35 520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the

intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

5 **VRC5517**

pVR1012x/s R5(SA-C)gp145(dCFI)dV123/h

10 The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to
15 BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V2, V3 loops (a.a.136-194, 297-325), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for
20 oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5518

pVR1012x/s R5(SA-C)gp145(dCFI)dV1234/h

25 The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to
30 BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1,V2,V3 ,V4 loops (a.a.136-194, 297-325, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer
35 oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

5 **VRC5519**

pVR1012x/s R5(SA-C)gp145(dCFI)dV2/h

10 The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V2 loop (a.a.156-194), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

20 **VRC5520**

pVR1012x/s R5(SA-C)gp145(dCFI)dV23/h

25 The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V2, V3 loops (a.a.156-194, 297-325), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially

functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5521

5 pVR1012x/s R5(SA-C)gp145(dCFI)dV234/h

The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V2, V3, V4 loops (a.a.156-194, 297-325, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5522

pVR1012x/s R5(SA-C)gp145(dCFI)dV24/h

25 The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V2, V4 loops (a.a.156-194, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially

functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5523

5 pVR1012x/s R5(SA-C)gp145(dCFI)dV3/h

The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression
10 in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks
15 the V3 loop (a.a.297-325), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC5524

pVR1012x/s R5(SA-C)gp145(dCFI)dV34/h

The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012
25 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains
30 polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V3, V4 loops (a.a. 297-325, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially

functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5525

5 pVR1012x/s R5(SA-C)gp145(dCFI)dV4/h

The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression
10 in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks
15 the V4 loop (a.a.385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

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VRC5526

pVR1012x/s R5(SA-C)gp145(dCFI)dV13/h

The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic
25 version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains
30 polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V3 loops (a.a.136-150, 297-325), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially

functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

VRC5527

5 pVR1012x/s R5(SA-C)gp145(dCFI)dV134/h

The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression
 10 in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks
 15 the V1, V3, V4 loops (a.a.136-150, 297-325, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is
 20 pVR1012x/s (VRC2000).

VRC5528

pVR1012x/s R5(SA-C)gp145(dCFI)dV124/h

25 The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream
 30 from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V2, V4 loops (a.a.136-150, 156-194, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2
 35 (a.a.577-605), and the intracellular region. Regions important for oligomer

formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

5 **VRC5529**

pVR1012x/s R5(SA-C)gp145(dCFI)dV14/h

The protein sequence of the envelope polyprotein (gp145delCFI) from 97ZA012 (R5-tropic, GenBank accession number AF286227) was used to create a synthetic version of the gene (Clade-C gp145delCFI) using codons optimized for expression in human cells. The nucleotide sequence R5gp145delCFI shows little homology to the gene 97ZA012, but the protein encoded is the same. The XbaI (18nt up-stream from ATG) to BamHI (1914 nt down-stream from ATG) fragment which contains polylinker at the 5' end, Kozak sequence and ATG was cloned into the XbaI to BamHI sites of pVR1012x/s backbone. The truncated envelope polyprotein lacks the V1, V4 loops (a.a.136-150, 385-399), the Fusion and Cleavage (F/CL) domains (a.a. 487-520), the Interspace (IS) between Heptad (H) 1 and 2 (a.a.577-605), and the intracellular region. Regions important for oligomer formation may be partially functional. Heptad(H) 1, Heptad 2 and their Interspace(IS) are required for oligomerization. The expression vector backbone is pVR1012x/s (VRC2000).

Gag-Pol Plasmids

VRC3900

pVR1012x/s Gag/h

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The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein to produce pGag(fs)Pol/h (VRC4200). The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene using codons optimized for expression in human cells. To produce a gene that

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expresses all of the gag proteins, the region encoding pol amino acids 77-1003 were deleted from Gag(fs)Pol/h to produce Gag/h. This construct also encodes most of the protease (98 amino acids) gene encoding amino acids 3-77. Gag/h is expressed from the pVR1012x/s vector backbone.

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VRC3901

pVR1012x/s SIV Gag/h

The protein sequence of the gag polyprotein (amino acid from 1-550) SIVmac239(GenBank accession number M33262) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the SIVMac239 gene, but the protein encoded is the same.

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15 **VRC4000**

pVR1012x/s Gag-Pol/h

The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. Gag-Pol/h is expressed from the pVR1012x/s vector backbone.

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VRC4001

pVR1012x/s SIVGag-Pol/h

5 Eukaryotic vector with humanized codons expressing the Gag-pol gene of SIVmac239. The Sal I–XbaI fragment of SIV gag(VRC3901) was inserted into Sal I–XbaI of SIV Pol(VRC4101) to create VRC4001.

VRC4100

pVR1012x/s Pol/h

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The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene using codons optimized for expression in human cells. To initiate translation at the beginning of Pol, a methionine codon was added to the 5'-end of the synthetic polymerase gene to create the Pol/h gene. Pol/h is expressed from the

15 pVR1012x/s (VRC2000) vector backbone.

VRC4101

pVR1012x/s SIV Pol/h

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The protein sequence of the pol polyprotein (amino acids 3-1017) from SIVmac239 (GenBank accession number M19921) was used to create a synthetic version of the pol gene using codons optimized for expression in human cells. To initiate translation at the beginning of Pol, a methionine codon was added to the 5'-end of the synthetic polymerase gene to create the Pol/h gene. The Protease (Pr) mutation is at pol amino acid 123 and is AAG->GGA or amino acids R->G. Reverse transcriptase(RT) mutation is at aa 352(GAC to CAT D(D to H) –RT) and Integrase mutation is at aa 788(GAC to GGC(D to A) –IN). Pol/h is expressed from the

25 pVR1012x/s (VRC2000) vector backbone.

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VRC4200

pVR1012x/s Gag(fs)Pol/h

35 The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the

gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone.

VRC4300

pVR1012 Gag-Pol(d delta RT delta IN)/h

Eukaryotic vector with humanized codons expressing the Gag and the frame shifted Pol genes of HIV HXB2 subtype B with deletions in Reverse transcriptase, and Integrase regions. The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express

the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag-pol amino acid 1209 and is ACT->CAT or amino acids D->A. Note: This vector is not in the pVR1012x/s backbone.

VRC4301

pVR1012x/s-Gag(FS)-Pol-delta RT IN-IRES-R5 gp157-Nef

For the Gag(FS)Pol delta RT delta IN/h portion, VRC4302 pVR1012x/s Gag(fs)Pol(delta PR delta/h was used for the protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid

771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag-pol amino acid 1209 and is ACT->CAT or amino acids D->A. This gene has been fused to an Internal Ribosomal Entry Site (IRES) and then fused to the R5gp157-Nef from VRC2200 pVR1012x/s R5gp157- NefDMHCDCD4/h in which the protein sequence of the envelope polyprotein (gp160) from HXB2 (X4-tropic, GenBank accession number K03455) was used to create a synthetic version of the gene (X4gp160/h) using codons optimized for expression in human cells. The nucleotide sequence X4gp160/h shows little homology to the HXB2 gene, but the protein encoded is the same with the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T. To produce an R5-tropic version of the envelope protein (R5gp160/h), the region encoding HIV-1 envelope polyprotein amino acids 275 to 361 from X4gp160/h (VRC3300) were replaced with the corresponding region from the BaL strain of HIV-1 (GeneBank accession number M68893, again using human preferred codons). The envelope-Nef fusion protein expressed from pR5gp157-Nef/h contains the first 820 amino acids from the HIV envelope glycoprotein (gp157) fused to the entire mutant Nef protein. The gene for gp157 was ligated in frame with the full-length mutant Nef gene from pNefDMHCDCD4/h (VRC3600) to produce pR5gp157- NefDMHCDCD4/h. The protein sequence of the Nef protein from HIV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. To disrupt the ability of Nef to limit both MHC class I and CD4 expression, point mutations were introduced into the Nef gene from pNef/h (VRC3500). The resulting amino acids substitutions in pNefDMHCDCD4/h are: P69A, P72A, P75A, P78A, D174A and D175A. R5gp157-NefDMHCDCD4/h is expressed from the pVR1012x/s (VRC2000) vector backbone.

VRC4302

pVR1012 Gag(delFS)Pol(delta PR delta RT delta IN)/h

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The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag

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proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag- pol amino acid 1209 and is ACT->CAT or amino acids D->A.

Note: This vector is not in the pVR1012x/s backbone.

VRC4303

pVR1012 SIV Gag(delFS)Pol(delta PR delta RT delta IN)/h

Eukaryotic vector with humanized codons expressing the Gag and the frame-shift-deleted Pol genes SIVmac239 with deletions in the Protease, Reverse transcriptase, and Integrase regions. The 5 of Ts from 3188 to 3192 of SIV gag-pol(VRC4001) were deleted to create VRC4303.

VRC4304

pVR1012 Gag (delFS)Pol delta PR delta RT delta IN/h

The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HIV-1 C clade(GenBank accession number U52953) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The

nucleotide sequence of the synthetic gag gene shows little homology to the HIV-1 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag- pol amino acid 1209 and is ACT->CAT or amino acids D->A. Note: This vector is not in the pVR1012x/s backbone.

VRC4305

25 pVR1012 Gag-A(delFS)Pol(delta PR delta RT delta IN)/h

Eukaryotic vector with humanized codons expressing the Gag and the frame shifted Pol genes of HIV HIV-1A clade with deletions in the Protease, Reverse transcriptase, and Integrase regions. VRC4305 pVR1012 Gag(-AdelFS)Pol(delta PR delta RT delta IN)/h The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HIV-1 A clade (GenBank accession number AF004885) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HIV-1 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally

cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag- pol amino acid 1209 and is ACT->CAT or amino acids D->A. Note: This vector is not in the pVR1012x/s backbone.

pVRC4306

pVR1012 Gag(delFS)Pol delta PR delta RT delta IN/Nef/h

The protein sequence of the Gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the Gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic Gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic Gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the Gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic Gag gene was ligated in frame with sequences encoding the Pol polyprotein. The protein sequence of the Pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the Pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the Gag-Pol

polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of Gag plus an additional 3 amino acids from Pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all Gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. The Protease (PR) mutation is at Gag-Pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at Gag-Pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at Gag- Pol amino acid 1209 and is ACT->CAT or amino acids D->A. The Nef/h gene was fused to downstream of Pol gene of Gag(delFS)Pol(delta PR delta RT delta IN). No loss or extra-amino acid was created by the fusion between Nef and Pol. The ATG of Nef was preserved. The protein sequence of the Nef protein from HTV-1 PV22 (GenBank accession number K02083) was used to create a synthetic version of the Nef gene (Nef/h) using codons optimized for expression in human cells. The nucleotide sequence Nef/h shows little homology to the viral gene, but the protein encoded is the same. Note: This vector is not in the pVR1012x/s backbone.

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VRC 4308

VRC4302-myr

pVR1012 Gag (delFS) Pol deltaPR deltaRT deltaIN deltaMyr/h

The myristoylation site was deleted from pVRC4302. The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons

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optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag-pol amino acid 1209 and is ACT->CAT or amino acids D->A. The myristylation site was deleted. Note: This vector is not in the pVR1012x/s backbone.

VRC 4309

pVR1012 Gag (delFS) Pol deltaPR deltaRT deltaIN delta Myr/Nef/h

The myristoylation site was deleted from pVRC4306. The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank

accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed
5 from the pVR1012x/s vector backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag-pol amino acid 1209 and is ACT->CAT or amino acids D->A. The stop codon TAG was removed and
10 synthetic B clade Nef (Genbank access number) was fusion to the 3' end of pol by PCR. Note: This vector is not in the pVR1012x/s backbone.

VRC 4310

pVR1012 Nef Gag (del fs) (del Myr) Pol (delta PR delta RT delta IN)/h

15 The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank
20 accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were
25 replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed from the pVR1012x/s vector
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backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag-pol amino acid 1209 and is ACT->CAT or amino acids D->A.

5 The stop codon TAG of synthetic B clade Nef (Genbank access number) gene was removed and was fused to the 5' end of gene gene by PCR. Note: This vector is not in the pVR1012x/s backbone.

VRC 4311

10 pVR1012 Gag-C(delFS)Pol(delta PR delta RT delta IN) Nef/h
VRC4304 + Clade C Nef

The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HIV-1 C clade (GenBank accession number U52953) was used to create a synthetic version

15 of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HIV-1 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The

20 synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To

25 create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The

30 substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol

35 amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN)

mutation is at gag-pol amino acid 1209 and is ACT->CAT or amino acids D->A. The stop codon TAG was removed and synthetic C clade Nef (Genbank accession number:U52953) was fusion to the 3' end of pol by PCR. Note:This vector is not in the pVR1012x/s backbone.

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VRC 4312

Gag (del fs) (del Myr) Nef Pol ΔPRΔRTΔIN/h

VRC-Myr-gag-Dnef-Dpol

(Eukaryotic vector with humanized codons expressing the Gag, truncated Nef, and truncated Pol proteins of HIV subtype B .ns.)

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This construct was derived from VRC4302. The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HXB2 (GenBank accession number K03455) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HXB2 gene, but the protein encoded is the same. The synthetic gag gene contains all of the mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding synthetic nef gene that 51 aa were deleted from 5'. 77 aa were deleted from 5' of pol polyprotein, and ligated with 3' of nef in which tag stop codon was deleted. The protein sequence of the pol polyprotein (amino acids 3-78 - 1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG-

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>GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag-pol amino acid 1209 and is ACT->CAT or amino acids D->A. Note: This vector is not in the pVR1012x/s backbone.

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VRC 4313

pVR1012 Gag Clade A (del fs)Pol(Δ PR Δ RT Δ IN)/h

VRC4305+ Clade A Nef

- 10 The protein sequence of the gag polyprotein (Pr55, amino acids 1-432) from HIV-1 A clade (GenBank accession number AF004885) was used to create a synthetic version of the gag gene using codons optimized for expression in human cells. The nucleotide sequence of the synthetic gag gene shows little homology to the HIV-1 gene, but the protein encoded is the same. The synthetic gag gene contains all of the
- 15 mature Gag proteins except for the last two that are normally cleaved from the carboxy-terminus of the gag polyprotein, p1 and p6 (amino acids 433-500). The synthetic gag gene was ligated in frame with sequences encoding the pol polyprotein. The protein sequence of the pol polyprotein (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) was used to create a synthetic version
- 20 of the pol gene (Pol/h) using codons optimized for expression in human cells. To create the possibility for translational frameshifting as means to express the gag-pol polyprotein, the synthetic coding region for the last four amino acids of the NC protein through the rest of gag plus an additional 3 amino acids from pol were replaced with the corresponding viral sequences (nucleotides 2074-2302 on the
- 25 HXB2 genome) from NL4-3 (GenBank accession number M19921). The substitution of viral for synthetic sequences both introduces the sites required for frameshifting and restores the ability to express all gag proteins, including p1 and p6. The deleted Frame Shifted (delFS) has 5 T nucleotides deleted between the Gag and Pol sequences. Gag(fs)Pol/h is expressed from the pVR1012x/s vector backbone. The Protease (PR) mutation is at gag-pol amino acid 553 and is AGG->GGC or amino acids R->G. The Reverse Transcriptase (RT) mutation is at gag-pol amino acid 771 and is GAC->CAC or amino acids D->H. The Integrase (IN) mutation is at gag-pol amino acid 1209 and is ACT->CAT or amino acids D->A. The stop codon TAG was removed and synthetic A clade Nef (Genbank accession
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number:AF069670) was fused to the 3' end of pol by PCR. Note: This vector is not in the pVR1012x/s backbone.

Examples

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Env

Immunogens

Plasmids expressing the CXCR4-tropic HIV-1 HXB2 Env were made synthetically with sequences designed to disrupt viral RNA structures that limit protein expression by using codons typically found in human cells. Briefly, the synthetic Env gene of HXB2 (GenBank accession number K03455) was generated in three fragments by assembling the overlapping synthetic oligonucleotides using PCR amplification. To produce a CCR5-tropic version of the HIV-1 envelope, the region encoding amino acids 275 to 361 of HXB2 (CXCR4-tropic) gp160 was replaced with CCR5-tropic HIV-1 BaL sequence (GenBank accession number M68893), which includes the V3 loop. Glycosylation mutants were generated by site-directed mutagenesis to replace asparagine with glutamic acid residues at seventeen conserved glycosylation sites between amino acids 88 and 448. To express truncation mutant Env proteins, stop codons were introduced after positions 752, 704, 680 or 592 to produce gp150, gp145, gp140, or gp128, respectively. The Env protein was further changed by deleting amino acids 503 to 537 and 593 to 619, which removes the cleavage site sequence, the fusion domain, and a part of the spacer between the two heptad repeats. The structures of the synthetic HIV envelope genes are shown (Fig. 1). The cDNAs were cloned in the expression vector pNGVL or pVR1012 under the control of the cytomegalovirus immediate-early enhancer, promoter, and first intron. The protein sequence was identical to HXB2 Env except for the following amino acid substitutions: F53L, N94D, K192S, I215N, A224T, A346D, P470L, T723I, and S745T.

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Expression of Envelope Proteins in Transfected Cells

293 cells (10^6) were plated in 60mm dishes. Cells were transfected on the following day with 2 μ g of plasmid using calcium phosphate (Graham FL, van der Eb AJ, 1973, Virology, 52:456-67). Cells were harvested 48 hours after transfection and lysed in buffer containing 50mM HEPES pH 7.0, 250mM NaCl and 0.5% NP40. The protein concentration in the lysates was determined using the Bradford reagent (Bio-Rad). Proteins (25 μ g) in lysates were separated by 7.5% SDS-PAGE

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and transferred to Immobilon-P membrane (Millipore, Bedford, MA). Env was detected by immunoprecipitation followed by Western blotting using polyclonal antibody against gp160 (Intracel, Rockville, MD).

Cell Surface Expression of Envelope by FACS Analysis

5 293 cells were harvested 48 hours after transfection and washed twice with phosphate-buffered saline (PBS) containing 1% bovine serum albumin and incubated for 30 minutes on ice with polyclonal immunoglobulin from an HIV-1 infected patient. The secondary antibody against human IgG conjugated with FITC (Jackson Immuno Research) was added, incubated for 30 minutes on ice, washed 3
10 times with PBS, and analyzed by flow cytometry (FACScan). The median fluorescent intensity values were derived using Cell Quant software.

DNA Injection in Mice

 Six week old, female BALB/c mice were injected intramuscularly with 100 µg of purified plasmid DNA suspended in 200 µl of normal saline. For each
15 plasmid DNA, a group of 4 mice was injected three times at intervals of two weeks. The mice were bled two weeks after the last injection, sera collected and stored at 4°C.

Quantitation of the Antibody Response

 Immunoprecipitation and Western blotting was used to detect the antibodies
20 that bind to native envelope proteins. Sera from immunized mice were used to immunoprecipitate gp160 from cell lysates of gp160-transfected 293 cells. Indicated dilutions were used to immunoprecipitate gp160 from the cell lysate (400 µg). Immunocomplexes were separated by 7.5% SDS-PAGE and analyzed by immunoblotting using the polyclonal antibody against gp160.

25 Analysis of CTL Response

 Spleens were removed aseptically and gently homogenized to a single cell suspension, washed, and resuspended to a final concentration of 5×10^7 cells/ml. Cells were incubated for 7 days in presence of IL-2 (10 U/ml) and either irradiated peptide-pulsed splenocytes from naive mice or an irradiated stable cell line
30 expressing the full length gp160 *BC-env/rev*, (Irwin MJ et al., 1994, J Virol, 68:5036-44). Three types of target cells were used: peptide-pulsed P815 cells (ATCC TIB64), BC10ME cells stably expressing gp160, and BC10ME cells (Collins JL, Patek PQ, Cohn M. Tumorigenicity and lysis by natural killers. *J Exp Med*, 1981; 153:89-106) pulsed with peptides derived from gp160 sequence. Target

cells were labeled with ^{51}Cr for 90 minutes and washed three times with RPMI-1640 with 10% FBS, 2mM glutamine, $5 \times 10^{-5}\text{M}$ β -mercaptoethanol and fungisone, 250units/ml, and resuspended in this media. Cytolytic activity was determined in triplicate samples using all different target cell dilutions in a 5-hour ^{51}Cr -release assay (Ohno T et al., 1997, Gene Ther, 4:361-6).

Gag and Pol

Development of Synthetic HIV-1 Gag-Pol Expression Vectors

The protein sequences of Gag (amino acids 1-432) from HXB2 (GenBank accession number K03455) and Pol (amino acids 3-1003) from NL4-3 (GenBank accession number M19921) were reverse translated by the GCG Package (Genetic Computer Group, Inc., Madison, WI) using codons expected for human cells. A 226-bp fragment spanning the frame shift site and the overlapping region of the two reading frames from NL4-3 were retained to allow expression of Gag and Gag-Pol precursor polyproteins in the same construct. 86 oligonucleotides covering 4325 DNA base pairs with 5' SalI and 3' EcoRI sites were purchased from GIBCO Life Technologies. Each of the oligonucleotides was 75 base-pairs with 25 nt of overlap. The codon optimized Gag-Pol gene (hGag-Pol) was assembled by PCR with Pwo (Boehringer Mannheim) and Turbo Pfu (Stratagene) high fidelity DNA polymerase. The PCR conditions were optimized with a PCR optimization kit (Stratagene) on a gradient Robocycler (Stratagene). Full-length synthetic Gag-Pol gene was cloned into the Sal I and blunted Bgl II site of the mammalian expression vector pNGVL-3 (Xu, Ling et al., 1998, Nature Medicine, 4:37-42) and confirmed by DNA sequencing. Three additional constructs were derived from the hGag-Pol gene. 5 Thymidines (Ts) in the frame shift site (FS) of the hGag-Pol gene were deleted (ΔFS) and the protease was inactivated by replacing AGG in protease to GGC (R42G) to create hGag-Pol $\Delta\text{FS}\Delta\text{Pr}$ (Hung, M, 1998, J Virol, 72:4819-4824; Loeb, DD et al., 1989, Nature 340:397-400). 432 amino acids of the NH₂- terminal of hGag-Pol gene were deleted and an ATG start codon was added to create the hPol gene. 925 amino acids of the COOH-terminal hGag-Pol were deleted to create the hGag gene. hGagPol $\Delta\text{FS}\Delta\text{Pr}$, hPol and hGag genes were expressed in the pNGVL-3 plasmid, derived by insertion of a polylinker into pVR1012. The plasmid expressing viral Gag-Pol, pCMV Δ 8.2 was a kind gift (Naldini, L et al., 1996, Science, 272:263-267).

Transient Transfection and Analysis of Expression

293T cells were maintained in Dulbecco's modified Eagle medium (DMEM; GIBCO-BRL), supplemented with 10% fetal bovine serum (FBS). Plasmid DNAs were purified with double cesium chloride sedimentation gradients. Approximately
5 3×10^6 293T cells were placed in a 10-cm dish one day before transfection. 10 μ g of pCMVdR8.2 plasmid (containing the viral gag-pol gene), or 5 μ g of pVR1012s (containing the codon altered genes), were used to transfect 293T cells, using the calcium phosphate method (Chen, C and H Okayama, 1987, Mol. Cell. Biol, 7:2745-2752). Three days after transfection, cell lysates were prepared with RIPA buffer
10 (Boehringer Mannheim, Indianapolis, IN) and separated by 4-15% gradient SDS-polyacrylamide gel electrophoresis (PAGE), then transferred onto an Immobilon P membrane (Millipore). Membranes were then incubated with anti HIV-1-IgG (AIDS Research and Reference Reagent Program), monoclonal anti-p24 (ICN), or rabbit anti-RT (Intracel, Rockville, MD). Bands were visualized using the ECL
15 Western blotting detection reagent (Amersham Pharmacia Biotech, Piscataway, NJ), as described by the manufacturer. Expression levels were determined using a phosphorimager.

Generation of Stably Transfected Cell Lines

hGag and hPol genes were individually subcloned into the Xho I and EcoRI
20 sites of a retroviral vector, pPGS-CITE-Neo. Three plasmid systems were used to produce recombinant retroviruses containing the hGag or hPol genes (Yang, Z et al., 1998, Science, 279:1034-1037). 48 hours after transfection, the supernatants were collected to transduce CT26 and BC10ME (Collins, JL, 1981, J Exp. Med, 153:89-106) which are syngeneic to Balb/C mice, and selected in 0.8 mg/ml of G418 two
25 days after infection. The positive clones were screened and confirmed by Western blotting and maintained in 10% FCS supplemented RPMI (GIBCO-BRL) with 0.5mg/ml G418.

DNA Vaccination of Mice

Female BALB/C mice, 6-8 weeks old, were used for immunogenicity
30 studies. For DNA vaccination, mice were immunized with 100 μ l (0.5 μ g/ml DNA, 0.9% NaCl) in the quadriceps muscle of each hind leg every two weeks for a total of 4 injections.

CTL Assay

Animals were sacrificed after the last immunization and spleens removed from both naïve and immunized mice using aseptic techniques. Splenic lymphocytes were harvested and the chromium release CTL assay was performed in triplicate as previously described (Ohno, T. et al., 1997, Gene Ther, 4:361-366). The peptides used for sensitizing cells are as follows: Two peptide mixtures from the Gag protein P17(88-115) and p24(62-76). Seven peptide mixtures from the Pol protein: 1) P66(175-189), 2) P66(179-193), 3) P66(183-197), 4) P66(187-201), 5) P66(223-237), 6) P66(227-241), 7) P66(367-381).

Measurement of Antibody Responses*Anti-p24 ELISA assays*

The anti-p24 ELISA assay was performed in Immulon ninety-six-well plates (Dynet Technologies Inc., Chantilly, VA). The plates were coated with 50 µl of purified recombinant HIV-1IIB p24 antigen (Intracel) at a concentration of 2 µg/ml in PBS buffer, pH 7.4 (GIBCO) with 0.05% sodium azide. Plates were washed 3x in PBS containing 3% BSA and 0.05% Tween20 (blocking buffer) and incubated for 2 hours. Mouse sera were serially diluted from 1:100 to 1:12,800 in blocking buffer, added to the p24-coated plates and incubated overnight at 4°C. Plates were then washed four times with PBS (0.05% Tween 20), and incubated with goat anti-mouse IgG (1:10,000 dilution, Sigma) for 2 hours at room temperature. Plates were washed four times, and then pNPP alkaline phosphatase substrate (75 µl; Sigma, St. Louis, MO) was added to each well. The reaction was stopped after one hour by addition of 0.5 N NaOH (25 µl). The plates were read on an ELISA reader at 405 nm, and titers were calculated at a cutoff optical density of 0.4.

HIV-1 immunoblotting

The strips containing HIV-1 proteins (Immunectics Inc., Cambridge, MA) were incubated with pooled mouse sera at a dilution of 1:25. Purified human anti-HIV IgG (AIDS Research and Reference Reagent Program, Rockville, MD) was used as a positive control. Bands were visualized using the ECL Western blotting detection reagent (Amersham Pharmacia Biotech, Piscataway, NJ).

Immunoprecipitation and Western blotting

Three days after transfection, hPol gene-transfected 293T cell lysates were prepared with RIPA buffer. The pooled mouse sera were diluted with immunoprecipitation (IP) buffer (100mM KCl, 2.5mM MgCl₂, 20mM HEPES, pH

7.9, 0.1% NP-40, 1mM DTT and proteinase inhibitors). After adding 10 µg of the cell lysate containing the HIV-1 Pol protein, the reactions were incubated overnight on a rotator at 4°C. 250 µl of Protein G and A Sepharose beads (10%V/V in IP buffer) were then added, and the reactions were incubated for 2 hours on a rotator at 4°C. The beads were washed four times with IP buffer, resuspended in 30 µl of 1X sample buffer and loaded onto SDS-PAGE. After transfer onto an Immobilon P membrane (Millipore), membranes were incubated with anti HIV-1-IgG (AIDS Research and Reference Reagent Program). Bands were visualized using the ECL Western blotting detection reagent (Amersham Pharmacia Biotech).

10 Prime/Boost Vaccination Strategy

Groups of guinea pigs (4/group) were immunized (primed) with the vectors listed below, 3 times at 2 week intervals. Two weeks after the third DNA immunization, blood was collected for immune analysis. 2-3 days after the blood collection, the animals received a boost with a corresponding adenoviral construct, AdApt (Sullivan, N. et al., 2000, Nature, 408:605-608). Blood was again collected 2 weeks after the adenoviral boost. Blood was tested for neutralizing antibodies and (using an ELISA) the presence of antibody against the envelope. The groups are summarized below.

	Prime	Adenoviral Boost
20	gp140delCFI (R5)	Adv gp140delCFI(R5)
	gp145delCFI (R5)	Adv gp145delCFI(R5)
	gp145delCFI (R5)	Adv gp140delCFI(R5)
	gp140delCFI (89.6P)	Adv gp140delCFI(89.6P)
	gp145delCFI (89.6P)	Adv gp145delCFI(89.6P)
25	gp145delCFI (89.6P)	Adv gp140delCFI(89.6P)
	Adv gp140delCFI (R5)	Adv gp140delCFI(R5)
	Adv gp145delCFI (R5)	Adv gp145delCFI(R5)
	Adv gp145delCFI (R5)	Adv gp140delCFI(R5)
	Adv gp140delCFI (89.6P)	Adv gp140delCFI(89.6P)
30	Adv gp145delCFI (89.6P)	Adv gp145delCFI(89.6P)
	Adv gp145delCFI (89.6P)	Adv gp140delCFI(89.6P)

Results

The level of antibody in the sera after adenoviral boost was 8- to 10-fold higher than the level of antibody present after 3 injections of DNA alone, in almost all of the cases. Neutralizing antibodies were detected in the sera of animals immunized with either gp145delCFI DNA or gp140delCFI DNA followed by Advgp140delCFI.

Neutralization was also observed in animals immunized with gp145delCFI DNA followed by Adv gp145delCFI, and in animals both primed and boosted with adenovirus.

Optimal neutralization was seen when animals were primed with gp145delCFI DNA followed by adenovirus expressing gp140delCFI in both clade B (R5-Bal strain) and 89.6P (Clade B, dual tropic strain).

All neutralizing activity was able to be blocked with the V3 peptide of the corresponding strains.

While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention. All figures, tables, and appendices, as well as patents, applications and publications referred to above are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a polynucleotide encoding a modified glycosylation or Δ CFI HIV Env, or combination thereof, encoding a plasmid taken from Table 1 (or its modified HIV Env insert), or encoding an analog of said plasmid or insert having at least 95% identity thereto.
2. The nucleic acid molecule of Claim 1 which is plasmid 2100 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
3. The nucleic acid molecule of Claim 1 which is plasmid 2200 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
4. The nucleic acid molecule of Claim 1 which is plasmid 2300 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
5. The nucleic acid molecule of Claim 1 which is plasmid 2302 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
6. The nucleic acid molecule of Claim 1 which is plasmid 2400 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
7. The nucleic acid molecule of Claim 1 which is plasmid 2700 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
8. The nucleic acid molecule of Claim 1 which is plasmid 2701 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
9. The nucleic acid molecule of Claim 1 which is plasmid 2702 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
10. The nucleic acid molecule of Claim 1 which is plasmid 2706 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
11. The nucleic acid molecule of Claim 1 which is plasmid 2707 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

12. The nucleic acid molecule of Claim 1 which is plasmid 2800 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
13. The nucleic acid molecule of Claim 1 which is plasmid 2801 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
14. The nucleic acid molecule of Claim 1 which is plasmid 2804 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
15. The nucleic acid molecule of Claim 1 which is plasmid 2805 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
16. The nucleic acid molecule of Claim 1 which is plasmid 2810 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
17. The nucleic acid molecule of Claim 1 which is plasmid 2811 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
18. The nucleic acid molecule of Claim 1 which is plasmid 2812 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
19. The nucleic acid molecule of Claim 1 which is plasmid 2813 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
20. The nucleic acid molecule of Claim 1 which is plasmid 2814 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
21. The nucleic acid molecule of Claim 1 which is plasmid 2820 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
22. The nucleic acid molecule of Claim 1 which is plasmid 2821 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

23. The nucleic acid molecule of Claim 1 which is plasmid 2822 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
24. The nucleic acid molecule of Claim 1 which is plasmid 2823 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
25. The nucleic acid molecule of Claim 1 which is plasmid 2824 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
26. The nucleic acid molecule of Claim 1 which is plasmid 2825 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
27. The nucleic acid molecule of Claim 1 which is plasmid 2826 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
28. The nucleic acid molecule of Claim 1 which is plasmid 2827 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
29. The nucleic acid molecule of Claim 1 which is plasmid 2828 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
30. The nucleic acid molecule of Claim 1 which is plasmid 2829 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
31. The nucleic acid molecule of Claim 1 which is plasmid 2830 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
32. The nucleic acid molecule of Claim 1 which is plasmid 2831 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.
33. The nucleic acid molecule of Claim 1 which is plasmid 2832 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

34. The nucleic acid molecule of Claim 1 which is plasmid 2833 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 35. The nucleic acid molecule of Claim 1 which is plasmid 2834 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

36. The nucleic acid molecule of Claim 1 which is plasmid 2835 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 37. The nucleic acid molecule of Claim 1 which is plasmid 2836 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 38. The nucleic acid molecule of Claim 1 which is plasmid 2837 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

39. The nucleic acid molecule of Claim 1 which is plasmid 2838 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 40. The nucleic acid molecule of Claim 1 which is plasmid 2839 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

41. The nucleic acid molecule of Claim 1 which is plasmid 2840 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 42. The nucleic acid molecule of Claim 1 which is plasmid 2841 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 43. The nucleic acid molecule of Claim 1 which is plasmid 2842 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

44. The nucleic acid molecule of Claim 1 which is plasmid 2843 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

45. The nucleic acid molecule of Claim 1 which is plasmid 2844 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 46. The nucleic acid molecule of Claim 1 which is plasmid 2845 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

47. The nucleic acid molecule of Claim 1 which is plasmid 2846 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 48. The nucleic acid molecule of Claim 1 which is plasmid 2847 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 49. The nucleic acid molecule of Claim 1 which is plasmid 2848 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

50. The nucleic acid molecule of Claim 1 which is plasmid 2849 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 51. The nucleic acid molecule of Claim 1 which is plasmid 2850 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

52. The nucleic acid molecule of Claim 1 which is plasmid 2851 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 53. The nucleic acid molecule of Claim 1 which is plasmid 2852 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 54. The nucleic acid molecule of Claim 1 which is plasmid 2853 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

55. The nucleic acid molecule of Claim 1 which is plasmid 2854 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

56. The nucleic acid molecule of Claim 1 which is plasmid 2860 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 57. The nucleic acid molecule of Claim 1 which is plasmid 2861 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

58. The nucleic acid molecule of Claim 1 which is plasmid 2862 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 59. The nucleic acid molecule of Claim 1 which is plasmid 2863 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 60. The nucleic acid molecule of Claim 1 which is plasmid 2864 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

61. The nucleic acid molecule of Claim 1 which is plasmid 2865 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 62. The nucleic acid molecule of Claim 1 which is plasmid 2866 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

63. The nucleic acid molecule of Claim 1 which is plasmid 2867 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 64. The nucleic acid molecule of Claim 1 which is plasmid 2868 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 65. The nucleic acid molecule of Claim 1 which is plasmid 2869 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

66. The nucleic acid molecule of Claim 1 which is plasmid 2870 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

67. The nucleic acid molecule of Claim 1 which is plasmid 2871 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 68. The nucleic acid molecule of Claim 1 which is plasmid 2872 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

69. The nucleic acid molecule of Claim 1 which is plasmid 2873 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 70. The nucleic acid molecule of Claim 1 which is plasmid 2874 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 71. The nucleic acid molecule of Claim 1 which is plasmid 2900 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

72. The nucleic acid molecule of Claim 1 which is plasmid 3000 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 73. The nucleic acid molecule of Claim 1 which is plasmid 3200 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

74. The nucleic acid molecule of Claim 1 which is plasmid 3201 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 75. The nucleic acid molecule of Claim 1 which is plasmid 3202 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 76. The nucleic acid molecule of Claim 1 which is plasmid 3203 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

77. The nucleic acid molecule of Claim 1 which is plasmid 3300 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

78. The nucleic acid molecule of Claim 1 which is plasmid 3301 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 79. The nucleic acid molecule of Claim 1 which is plasmid 3400 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

80. The nucleic acid molecule of Claim 1 which is plasmid 3401 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 81. The nucleic acid molecule of Claim 1 which is plasmid 3500 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

82. The nucleic acid molecule of Claim 1 which is plasmid 3600 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

83. The nucleic acid molecule of Claim 1 which is plasmid 3700 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 84. The nucleic acid molecule of Claim 1 which is plasmid 3800 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

85. The nucleic acid molecule of Claim 1 which is plasmid 5200 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 86. The nucleic acid molecule of Claim 1 which is plasmid 5201 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

87. The nucleic acid molecule of Claim 1 which is plasmid 5202 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 88. The nucleic acid molecule of Claim 1 which is plasmid 5203 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

89. The nucleic acid molecule of Claim 1 which is plasmid 5300 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 90. The nucleic acid molecule of Claim 1 which is plasmid 5301 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

91. The nucleic acid molecule of Claim 1 which is plasmid 5303 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 92. The nucleic acid molecule of Claim 1 which is plasmid 5304 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 93. The nucleic acid molecule of Claim 1 which is plasmid 5305 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

94. The nucleic acid molecule of Claim 1 which is plasmid 5306 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 95. The nucleic acid molecule of Claim 1 which is plasmid 5307 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

96. The nucleic acid molecule of Claim 1 which is plasmid 5308 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 97. The nucleic acid molecule of Claim 1 which is plasmid 5309 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 98. The nucleic acid molecule of Claim 1 which is plasmid 5350 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

99. The nucleic acid molecule of Claim 1 which is plasmid 5351 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

100. The nucleic acid molecule of Claim 1 which is plasmid 5352 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 101. The nucleic acid molecule of Claim 1 which is plasmid 5353 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

102. The nucleic acid molecule of Claim 1 which is plasmid 5354 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 103. The nucleic acid molecule of Claim 1 which is plasmid 5355 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 104. The nucleic acid molecule of Claim 1 which is plasmid 5356 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

105. The nucleic acid molecule of Claim 1 which is plasmid 5357 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 106. The nucleic acid molecule of Claim 1 which is plasmid 5358 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

107. The nucleic acid molecule of Claim 1 which is plasmid 5359 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 108. The nucleic acid molecule of Claim 1 which is plasmid 5360 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 109. The nucleic acid molecule of Claim 1 which is plasmid 5361 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

110. The nucleic acid molecule of Claim 1 which is plasmid 5362 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

111. The nucleic acid molecule of Claim 1 which is plasmid 5363 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 112. The nucleic acid molecule of Claim 1 which is plasmid 5364 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

113. The nucleic acid molecule of Claim 1 which is plasmid 5365 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 114. The nucleic acid molecule of Claim 1 which is plasmid 5366 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 115. The nucleic acid molecule of Claim 1 which is plasmid 5367 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

116. The nucleic acid molecule of Claim 1 which is plasmid 5368 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 117. The nucleic acid molecule of Claim 1 which is plasmid 5369 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

118. The nucleic acid molecule of Claim 1 which is plasmid 5370 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 119. The nucleic acid molecule of Claim 1 which is plasmid 5371 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 120. The nucleic acid molecule of Claim 1 which is plasmid 5372 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

121. The nucleic acid molecule of Claim 1 which is plasmid 5373 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

122. The nucleic acid molecule of Claim 1 which is plasmid 5374 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 123. The nucleic acid molecule of Claim 1 which is plasmid 5375 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

124. The nucleic acid molecule of Claim 1 which is plasmid 5376 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 125. The nucleic acid molecule of Claim 1 which is plasmid 5377 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

126. The nucleic acid molecule of Claim 1 which is plasmid 5378 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 127. The nucleic acid molecule of Claim 1 which is plasmid 5379 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 128. The nucleic acid molecule of Claim 1 which is plasmid 5500 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

129. The nucleic acid molecule of Claim 1 which is plasmid 5501 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 130. The nucleic acid molecule of Claim 1 which is plasmid 5502 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 131. The nucleic acid molecule of Claim 1 which is plasmid 5503 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

132. The nucleic acid molecule of Claim 1 which is plasmid 5504 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

133. The nucleic acid molecule of Claim 1 which is plasmid 5505 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 134. The nucleic acid molecule of Claim 1 which is plasmid 5506 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

135. The nucleic acid molecule of Claim 1 which is plasmid 5507 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 136. The nucleic acid molecule of Claim 1 which is plasmid 5508 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 137. The nucleic acid molecule of Claim 1 which is plasmid 5509 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

138. The nucleic acid molecule of Claim 1 which is plasmid 5510 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 139. The nucleic acid molecule of Claim 1 which is plasmid 5511 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

140. The nucleic acid molecule of Claim 1 which is plasmid 5512 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 141. The nucleic acid molecule of Claim 1 which is plasmid 5513 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 142. The nucleic acid molecule of Claim 1 which is plasmid 5514 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

143. The nucleic acid molecule of Claim 1 which is plasmid 5515 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

144. The nucleic acid molecule of Claim 1 which is plasmid 5516 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 145. The nucleic acid molecule of Claim 1 which is plasmid 5517 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

146. The nucleic acid molecule of Claim 1 which is plasmid 5518 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 147. The nucleic acid molecule of Claim 1 which is plasmid 5519 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

148. The nucleic acid molecule of Claim 1 which is plasmid 5520 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 149. The nucleic acid molecule of Claim 1 which is plasmid 5521 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 150. The nucleic acid molecule of Claim 1 which is plasmid 5522 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

151. The nucleic acid molecule of Claim 1 which is plasmid 5523 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 152. The nucleic acid molecule of Claim 1 which is plasmid 5524 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 153. The nucleic acid molecule of Claim 1 which is plasmid 5525 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

154. The nucleic acid molecule of Claim 1 which is plasmid 5526 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

155. The nucleic acid molecule of Claim 1 which is plasmid 5527 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 156. The nucleic acid molecule of Claim 1 which is plasmid 5528 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

157. The nucleic acid molecule of Claim 1 which is plasmid 5529 (or its modified HIV Env insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 158. A nucleic acid molecule comprising a polynucleotide encoding in a continuous open reading frame a modified HIV Gag-Pol or Gag-Pol-Nef fusion protein, encoding a plasmid taken from Table 1 (or its modified HIV Gag, Pol, or Nef insert), or encoding an analog of said plasmid or insert having at least 95% identity thereto.

15 159. The nucleic acid molecule of Claim 158 which is plasmid 3900 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 160. The nucleic acid molecule of Claim 158 which is plasmid 3901 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

161. The nucleic acid molecule of Claim 158 which is plasmid 4000 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 162. The nucleic acid molecule of Claim 158 which is plasmid 4001 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

163. The nucleic acid molecule of Claim 158 which is plasmid 4100 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 164. The nucleic acid molecule of Claim 158 which is plasmid 4101 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

35 165. The nucleic acid molecule of Claim 158 which is plasmid 4200 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

166. The nucleic acid molecule of Claim 158 which is plasmid 4300 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 167. The nucleic acid molecule of Claim 158 which is plasmid 4301 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

168. The nucleic acid molecule of Claim 158 which is plasmid 4302 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

10 169. The nucleic acid molecule of Claim 158 which is plasmid 4303 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

15 170. The nucleic acid molecule of Claim 158 which is plasmid 4304 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

171. The nucleic acid molecule of Claim 158 which is plasmid 4305 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

20 172. The nucleic acid molecule of Claim 158 which is plasmid 4306 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

173. The nucleic acid molecule of Claim 158 which is plasmid 4308 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

25 174. The nucleic acid molecule of Claim 158 which is plasmid 4309 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

30 175. The nucleic acid molecule of Claim 158 which is plasmid 4310 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

176. The nucleic acid molecule of Claim 158 which is plasmid 4311 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

177. The nucleic acid molecule of Claim 158 which is plasmid 4312 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

5 178. The nucleic acid molecule of Claim 158 which is plasmid 4313 (or its modified HIV Gag, Pol, or Nef insert) or an analog of said plasmid or insert having at least 95% identity thereto.

179. A modified HIV Env, Gag, Pol, or Nef encoded by the nucleic acid molecule of any of Claims 1-178.

10 180. The nucleic acid molecule of any of Claims 1-178 further comprising a backbone, wherein said backbone is a plasmid vector.

181. The nucleic acid molecule of any of Claims 1-178 further comprising a backbone, wherein said backbone is an adenoviral vector.

15 182. A nucleic acid molecule comprising a polynucleotide encoding a modified glycosylation or Δ CFI HIV Env, or combination thereof, encoding a modified HIV Env insert taken from a plasmid taken from Table 1, or encoding an analog of said insert having at least 95% identity thereto, and further encoding in a continuous open reading frame a modified HIV Gag-Pol or Gag-Pol-Nef fusion protein, encoding a modified HIV Gag, Pol, or Nef insert taken from a plasmid taken from Table 1, or encoding an analog of said insert having at least 95% identity thereto.

20 183. A pharmaceutical composition comprising at least one nucleic acid molecule of any of Claims 1-178 and a pharmaceutically acceptable solution in a therapeutically effective dose

25 184. A vaccine composition comprising at least one nucleic acid molecule of any of Claims 1-178 and a pharmaceutically acceptable solution in a prophylactically effective dose.

185. A pharmaceutical composition comprising at least one modified HIV Env, Gag, Pol, or Nef of Claim 179 and a pharmaceutically acceptable solution in a therapeutically effective dose.

30 186. A vaccine composition comprising at least one modified HIV Env, Gag, Pol, or Nef of Claim 179 and a pharmaceutically acceptable solution in a prophylactically effective dose.

187. The composition of any of Claims 183-186 additionally comprising an adjuvant.

35 188. The composition of Claim 187 wherein said adjuvant is a cytokine.

189. The composition of Claim 183 or 184 which combines plasmid 4306 (gag-pol-nef) and plasmid 2827 (env).

190. The composition of Claim 183 or 184 which combines plasmid 4306 (gag-pol-nef Clade B), plasmid 2827 (env Clade B), plasmid 4311 (gag-pol-nef Clade C), and plasmid 5509 (env Clade C).

191. A method comprising the step of administering by genetic immunization a nucleic acid molecule of any of Claims 1-178 to a host to ameliorate the symptoms of AIDS or HIV infection.

192. A method comprising the step of administering by genetic immunization a nucleic acid molecule of any of Claims 1-178 to a host to generate an antibody or CTL response against native HIV Env, Gag, Pol, or Nef.

193. A method comprising the step of administering by protein immunization a modified HIV Env, Gag, Pol, or Nef of Claim 179 to a host to ameliorate the symptoms of AIDS or HIV infection.

194. A method comprising the step of administering by protein immunization a modified HIV Env, Gag, Pol, or Nef of Claim 179 to a host to generate an antibody or CTL response against native HIV Env, Gag, Pol, or Nef.

195. The method of any of Claims 191-194 wherein said step is a primary immunization.

196. The method of any of Claims 191-194 wherein said step is a boost.

197. The method of Claims 191 or 192 wherein said step is a primary immunization with a plasmid vector.

198. The method of Claims 191 or 192 wherein said step is a boost with an adenoviral vector.

199. The method of Claims 191 or 192 wherein said step is a primary immunization with a plasmid vector expressing gp145delCFI and further comprising a step which is a boost with an adenoviral vector expressing gp140delCFI.

200. A nucleic acid molecule of any of Claims 1-178 for use by genetic immunization in amelioration of the symptoms of AIDS or HIV infection.

201. A nucleic acid molecule of any of Claims 1-178 for use by genetic immunization in generation of an antibody or CTL response against native HIV Env, Gag, Pol, or Nef.

202. A modified HIV Env, Gag, Pol, or Nef of Claim 179 for use by protein immunization in amelioration of the symptoms of AIDS or HIV infection.

203. A modified HIV Env, Gag, Pol, or Nef of Claim 179 for use by protein immunization in generation of an antibody or CTL response against native HIV Env, Gag, Pol, or Nef.

5 204. A nucleic acid molecule comprising a polynucleotide encoding a modified Δ CFI HIV Env or encoding in a continuous open reading frame a modified HIV Gag-Pol or Gag-Pol-Nef fusion protein, or a combination thereof, applied to any HIV clade or strain.

10 205. A nucleic acid molecule comprising a polynucleotide encoding a modified Δ CFI HIV Env or encoding in a continuous open reading frame a modified HIV Gag-Pol or Gag-Pol-Nef fusion protein, or a combination thereof, applied to related proteins of other viruses.

15 206. A nucleic acid molecule comprising a polynucleotide encoding a modified viral membrane fusion protein with deletions in sufficient domains to expose a core protein for optimal antigen presentation, wherein the viral membrane fusion protein is defined by a central triple stranded coiled coil followed by a disulfide-bonded loop that reverses the chain direction and connects to an α helix packed antiparallel to the core helices.

20 207. A nucleic acid molecule comprising a polynucleotide encoding in a continuous open reading frame a modified viral protein, wherein the viral protein is transcribed in native form by translational frame shifting in a non-equivalent ratio or through a separate open reading frame.

Figure A

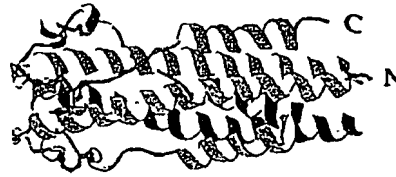
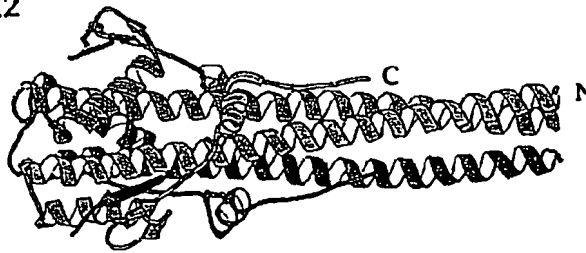
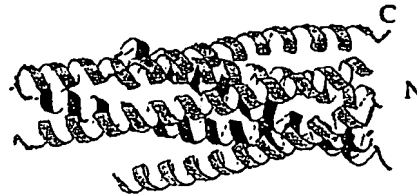
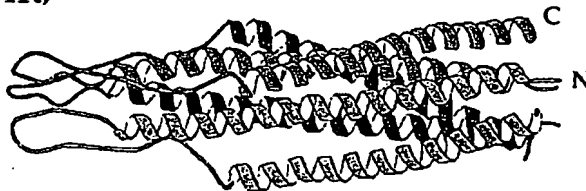
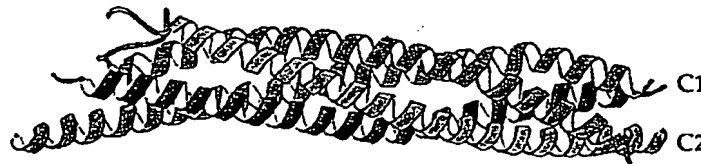
A Ebola GP2**B MoMuLv Mo-55****C low-pH-treated
influenza HA2****D protease resistant
core of HIV gp41****E SIV gp41 (NMR)****F SNAREs**

Figure I

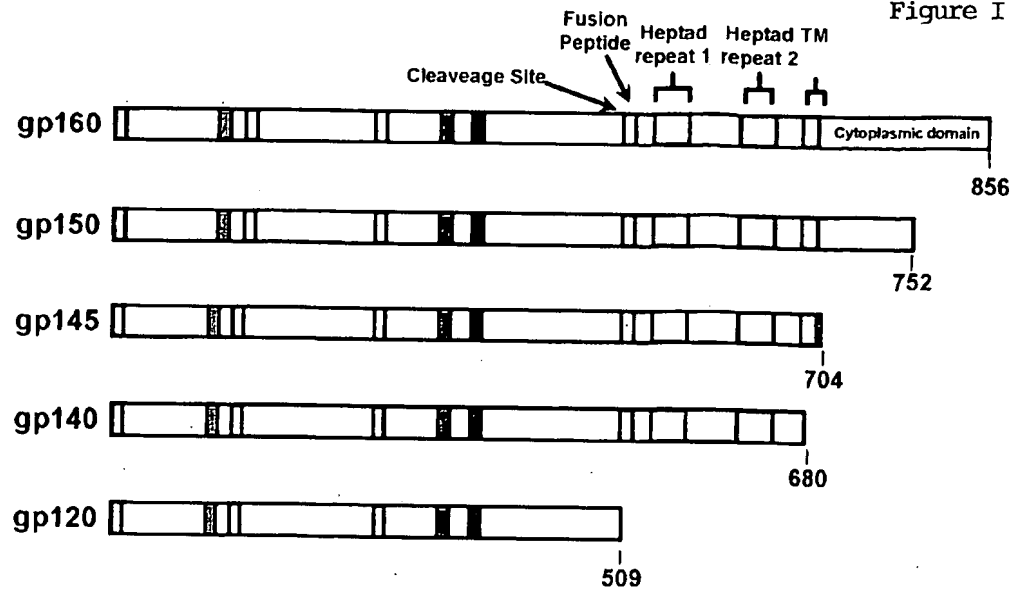
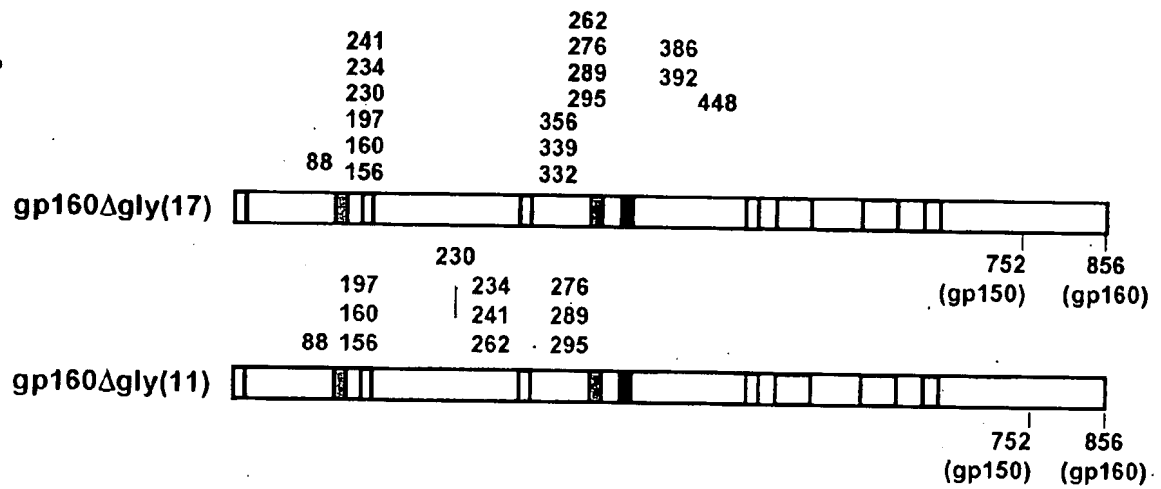
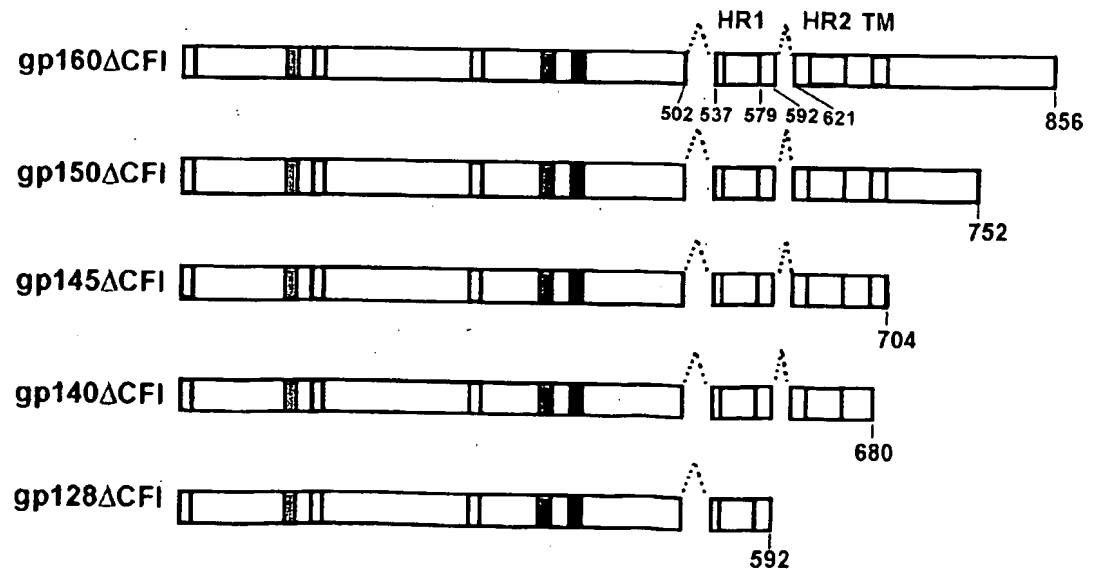
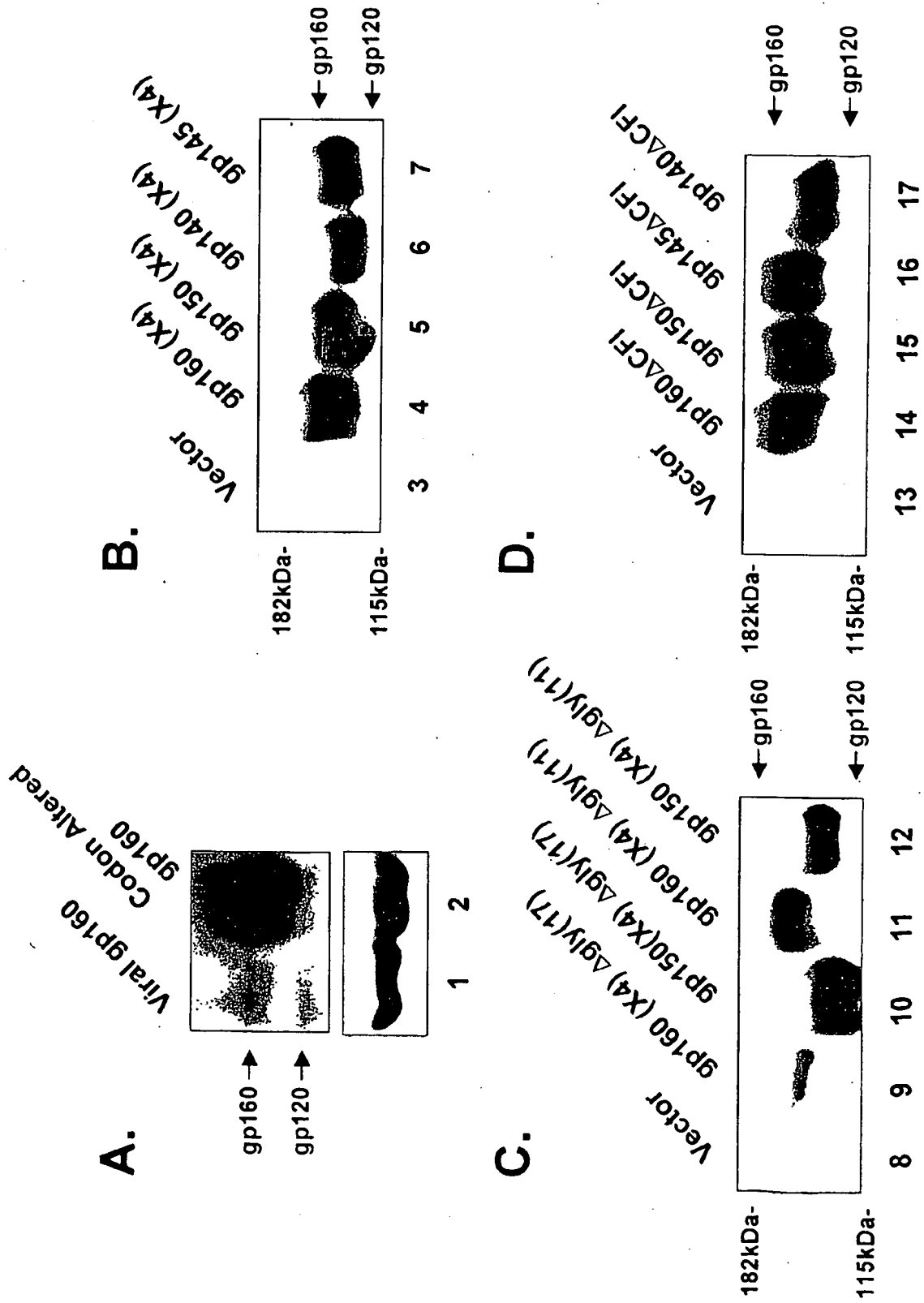
A.**B.****C.**

Figure II



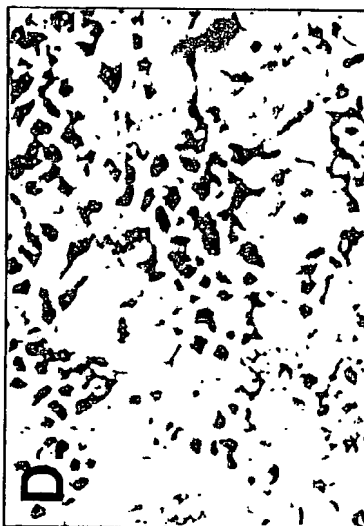


Figure IV

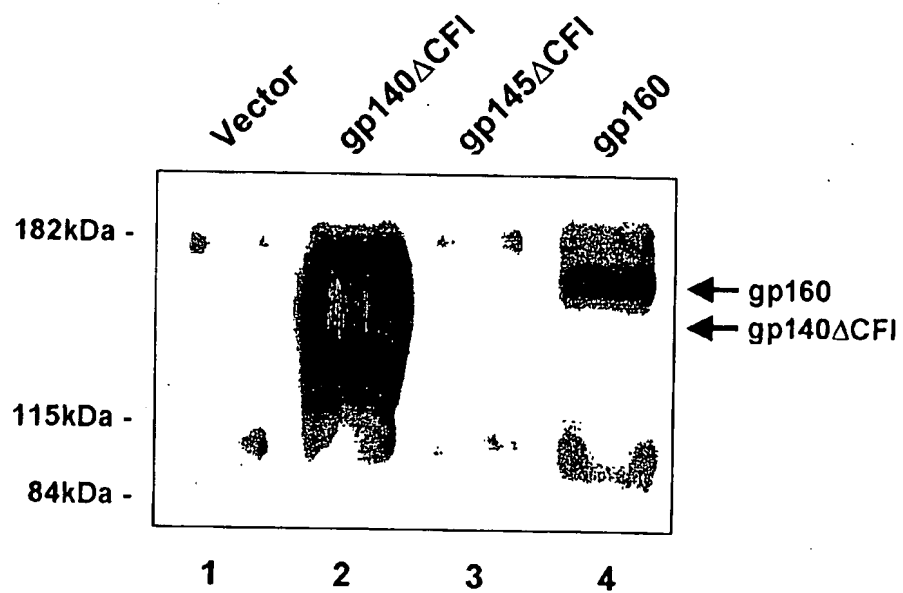


Figure V

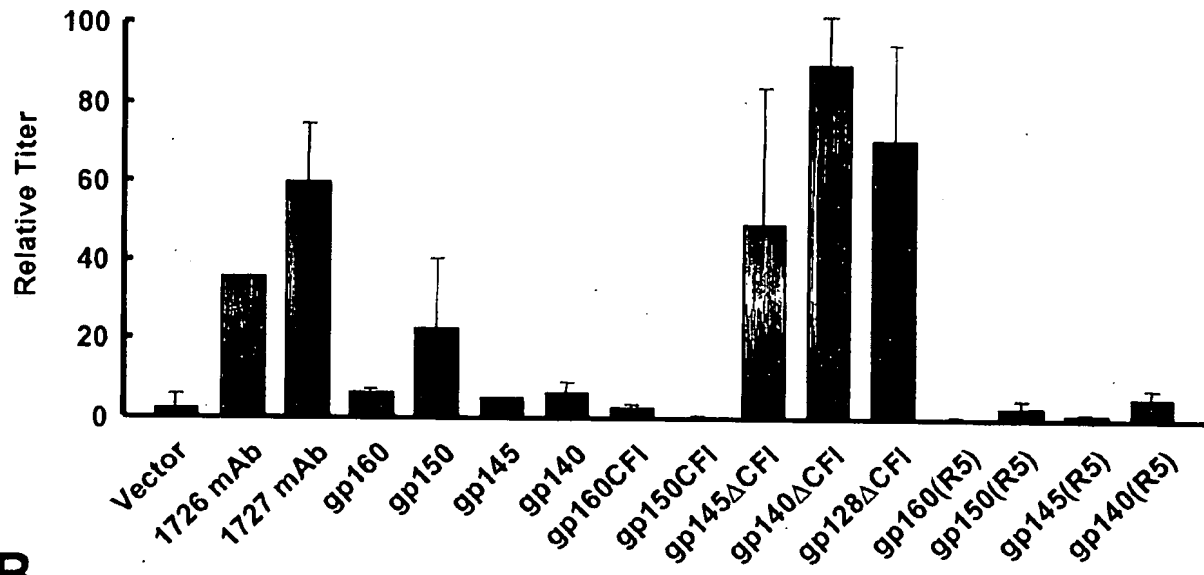
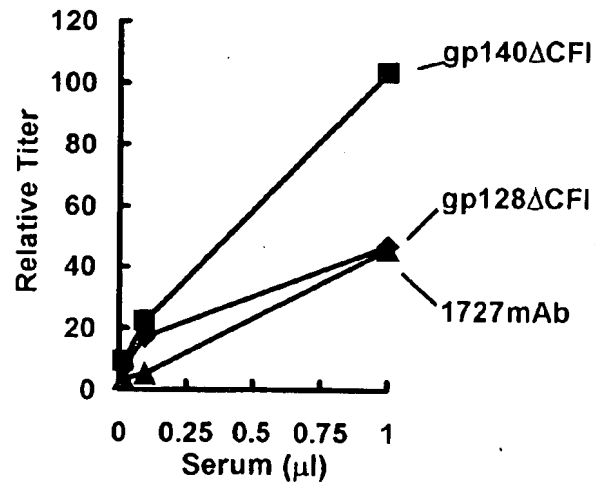
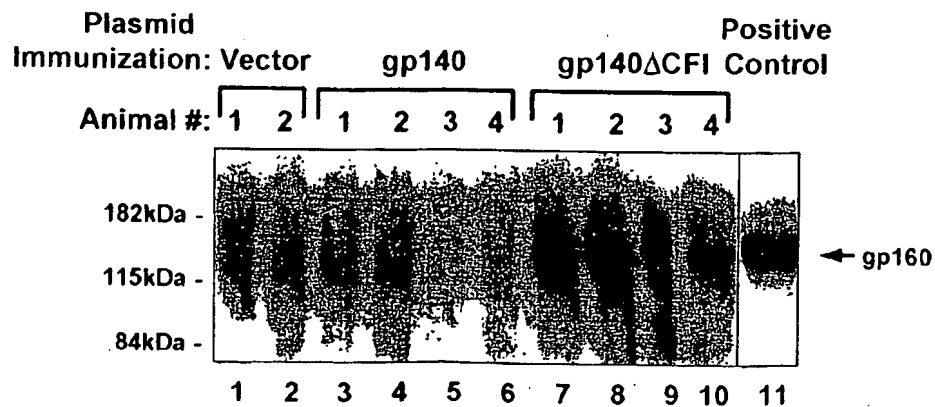
A.**B.****C.**

Figure VI

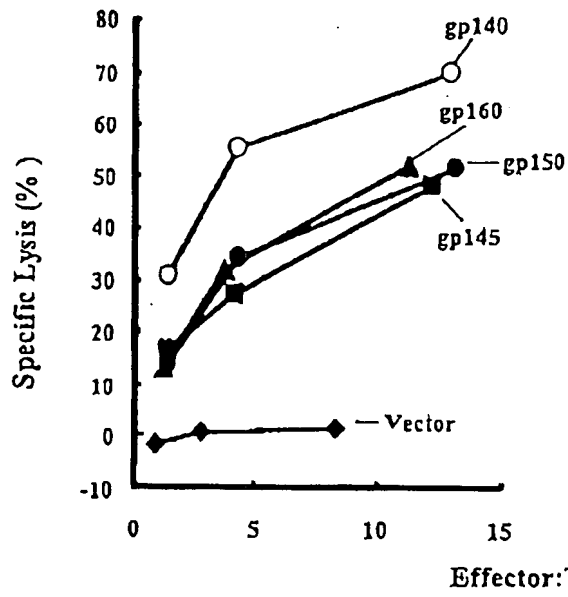
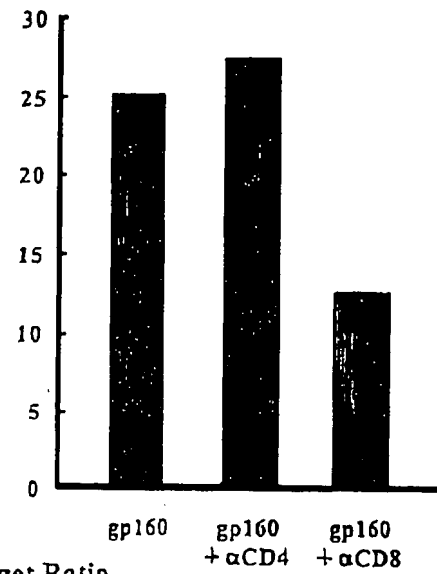
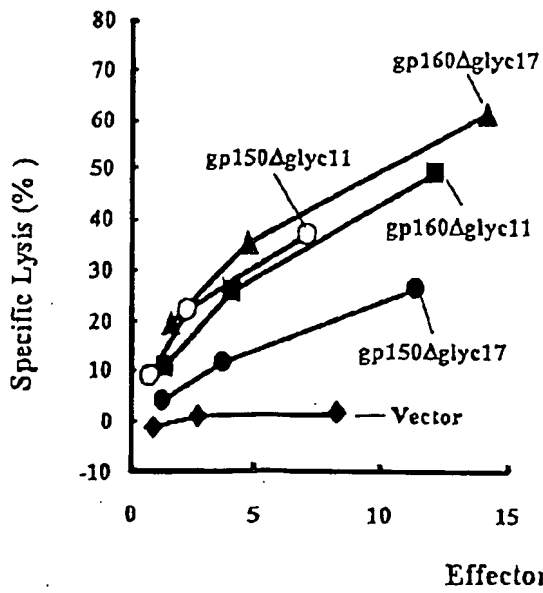
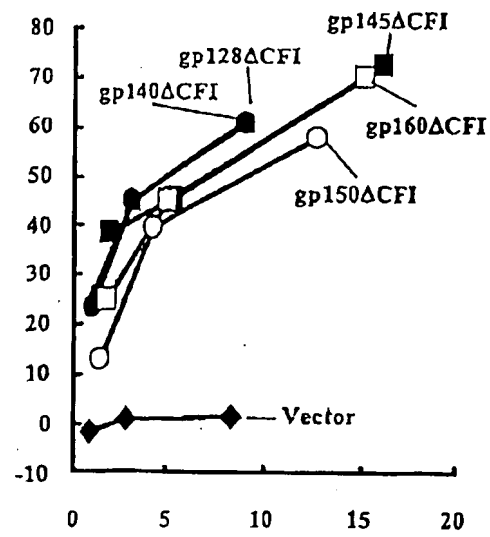
A.**B.****C.****D.**

Figure VII

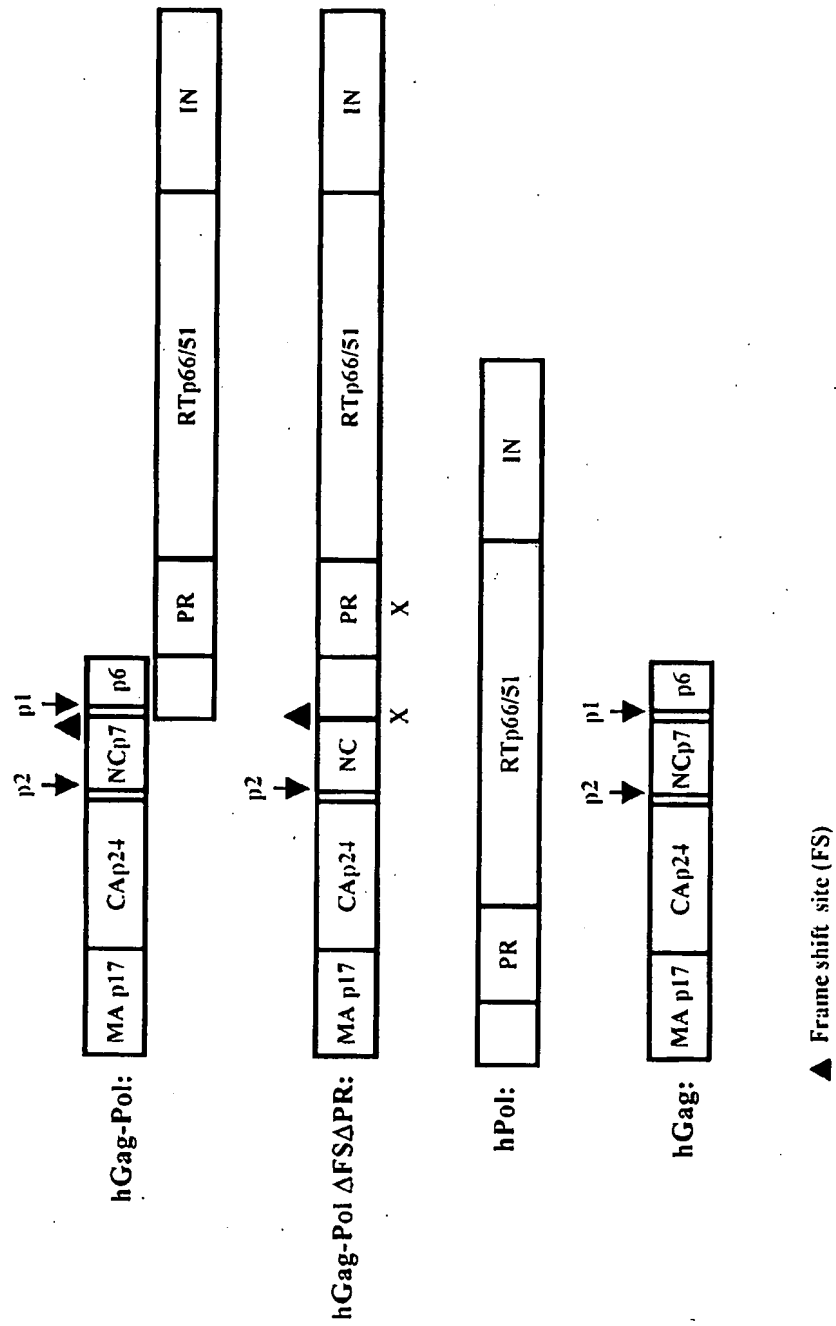
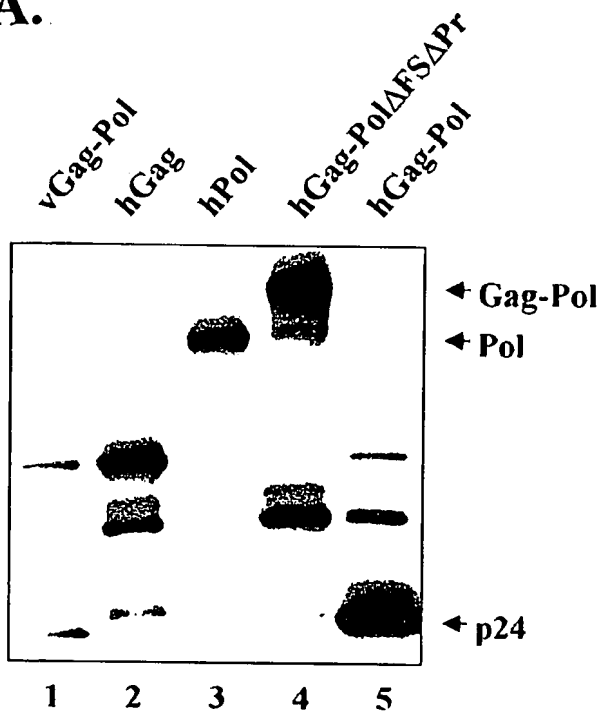
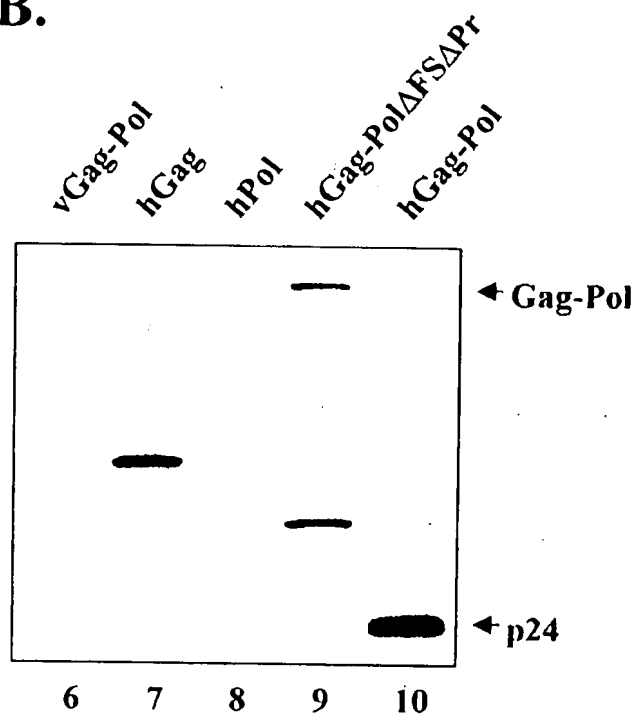


Figure VIII

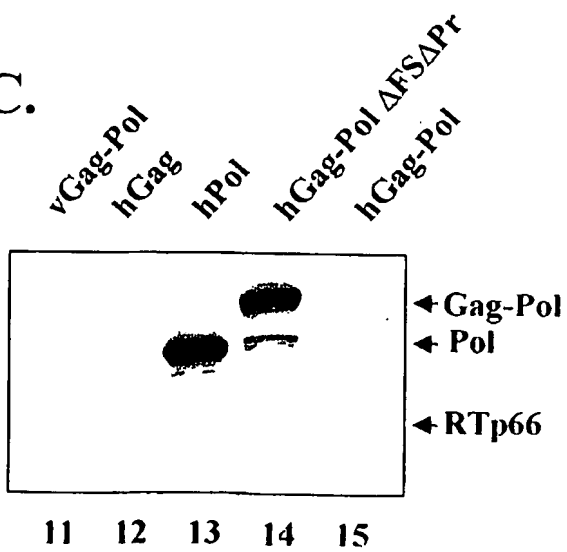
A.



B.



C.



D.

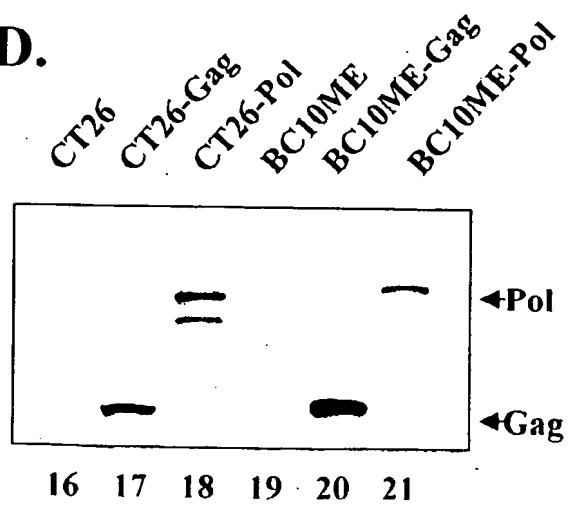
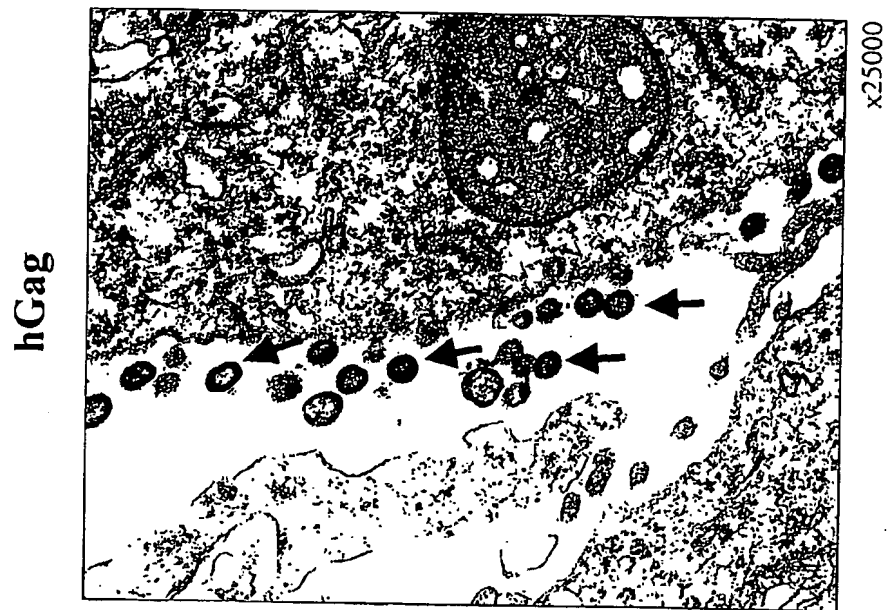
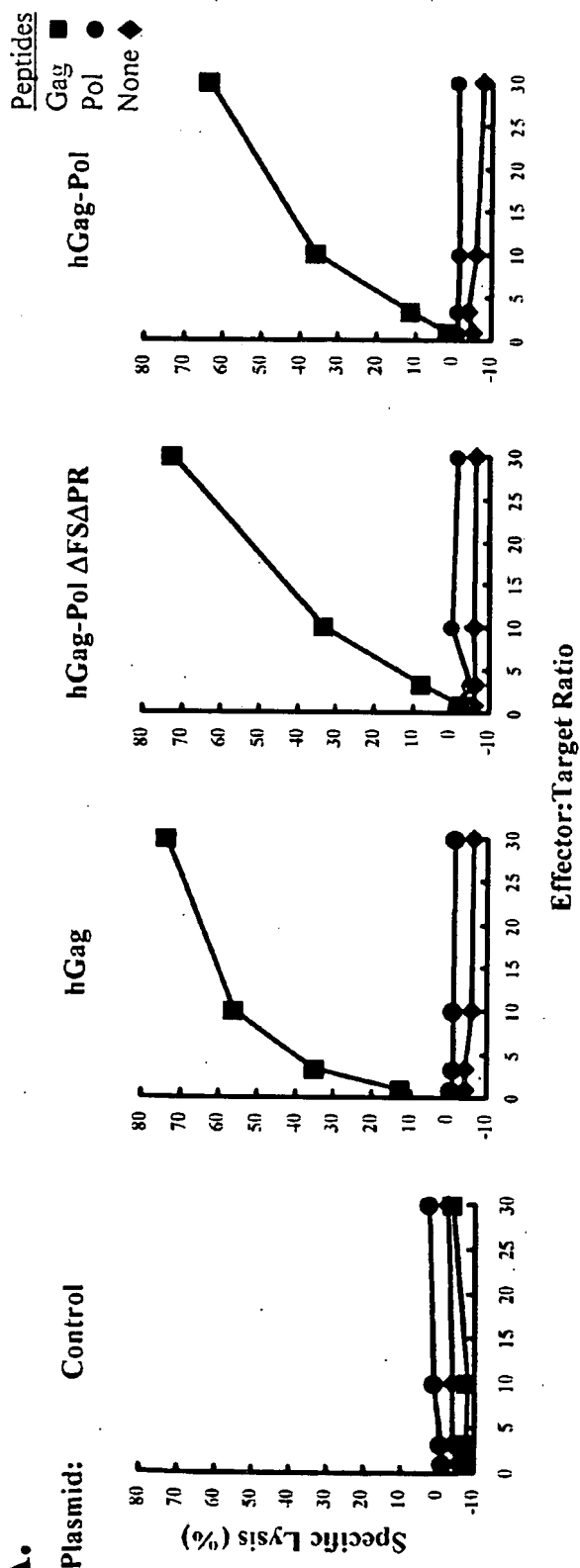


Figure IX



A.



B.

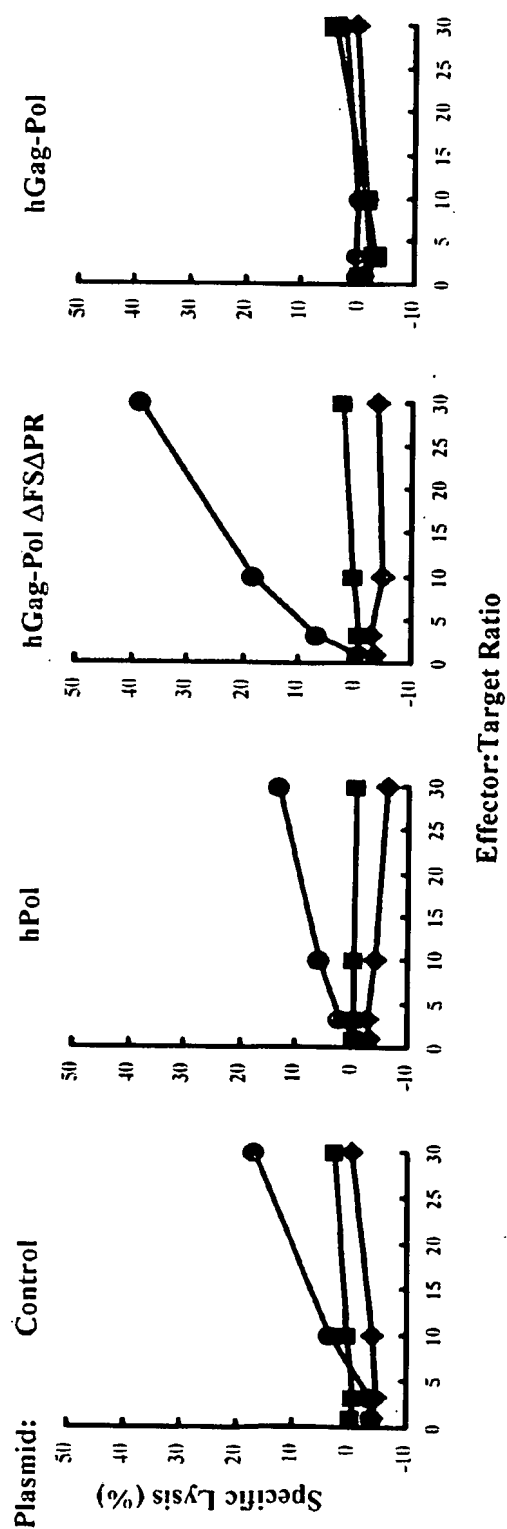


Figure X

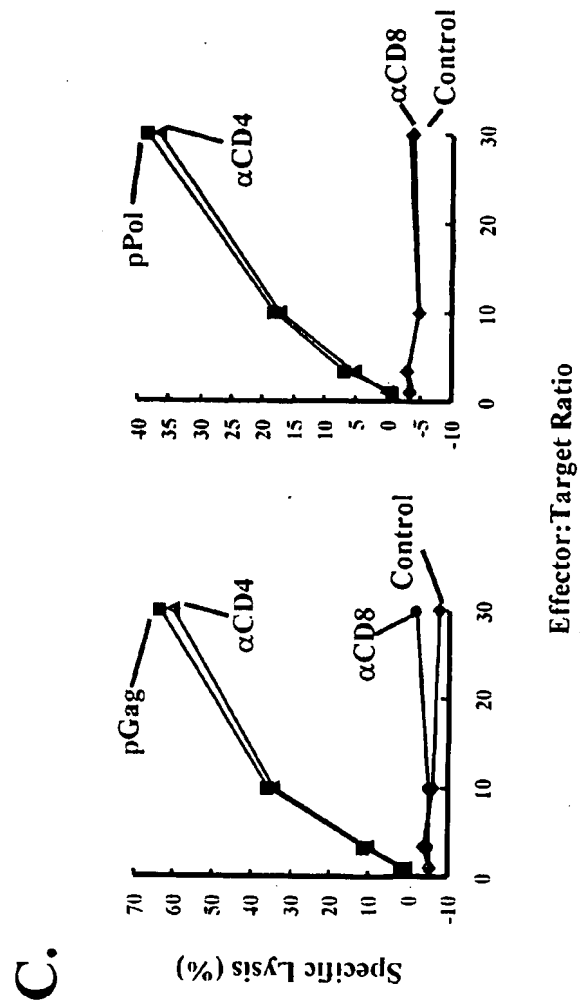
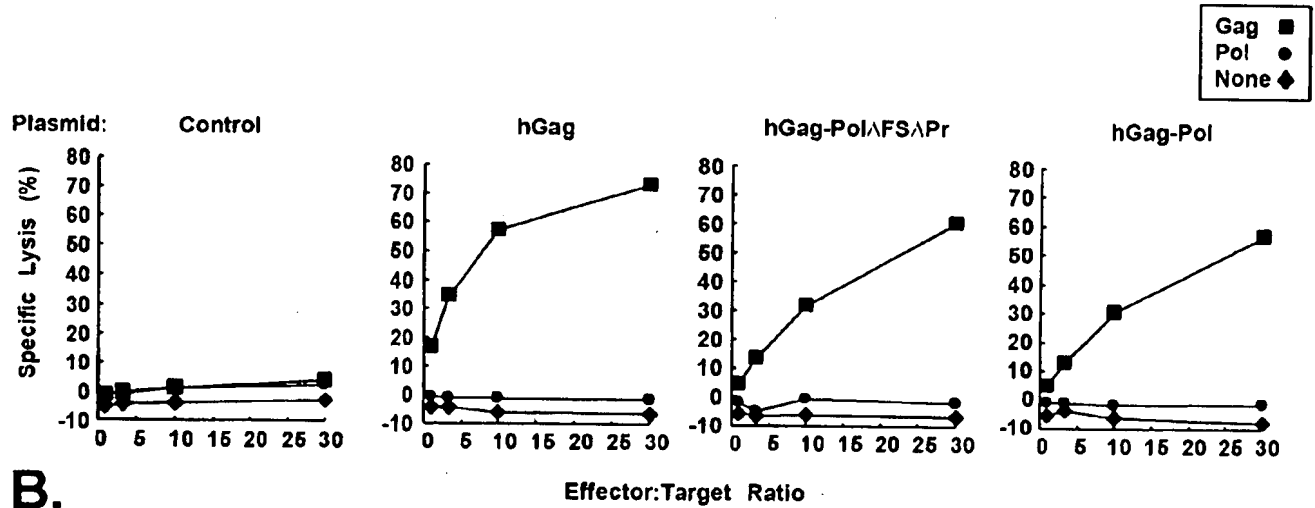
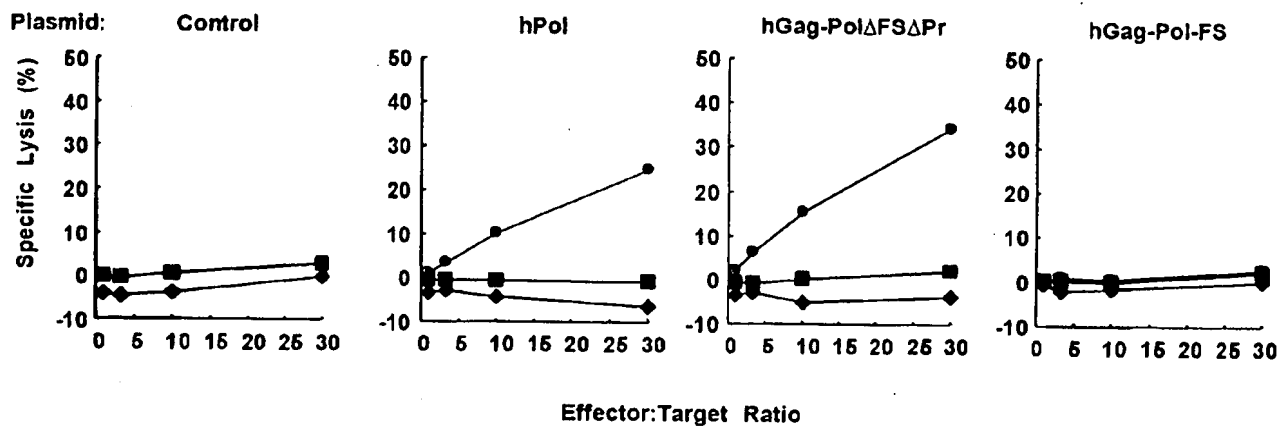
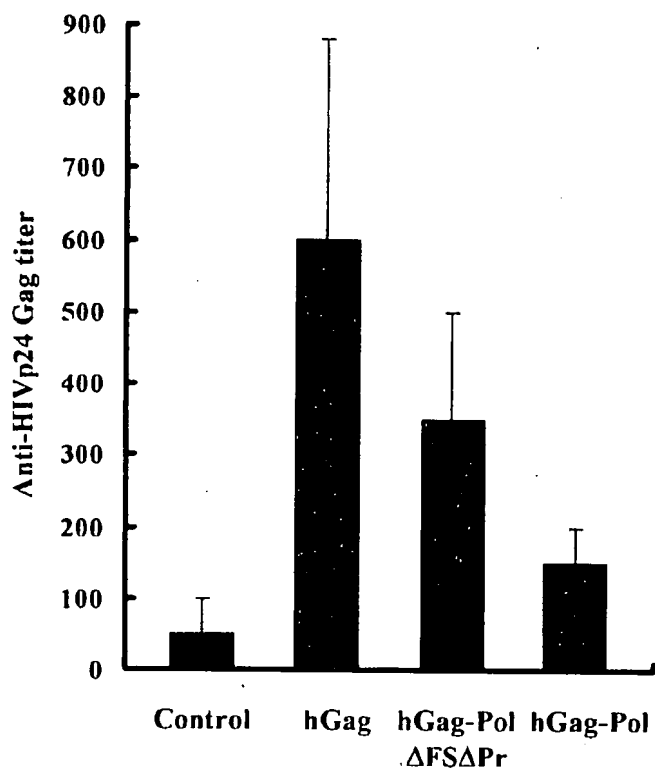
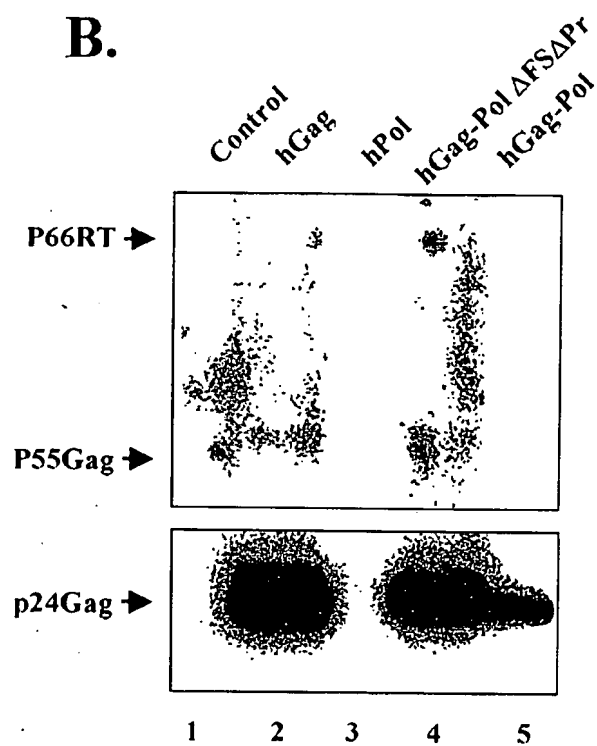
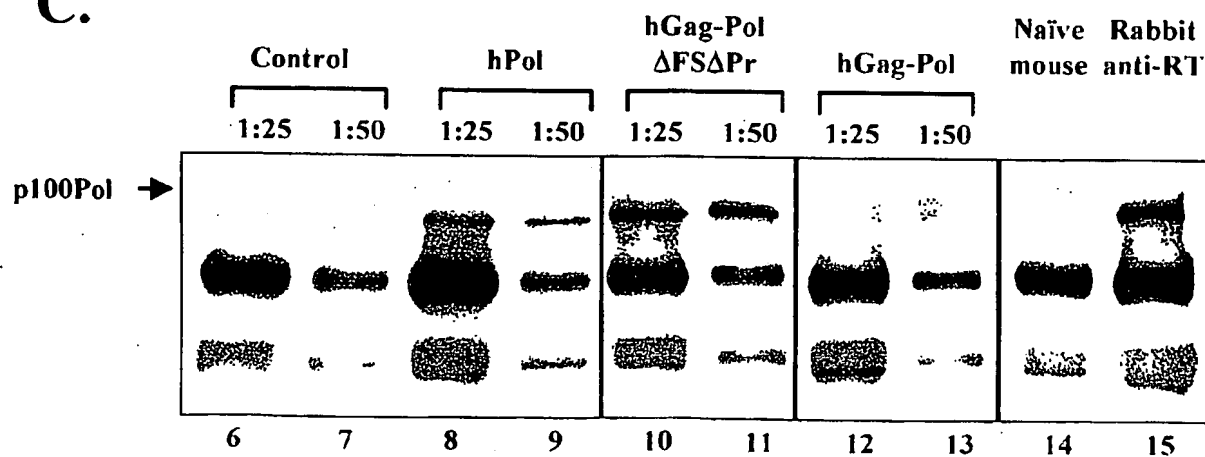


Figure XI

A.**B.**

A.**B.****C.**

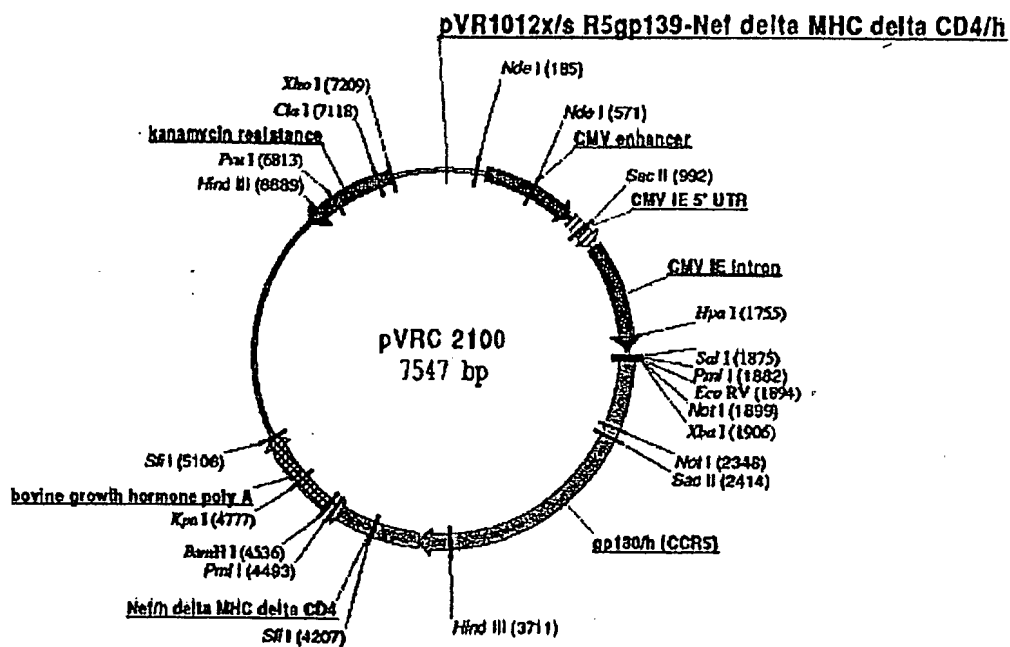


Figure 1

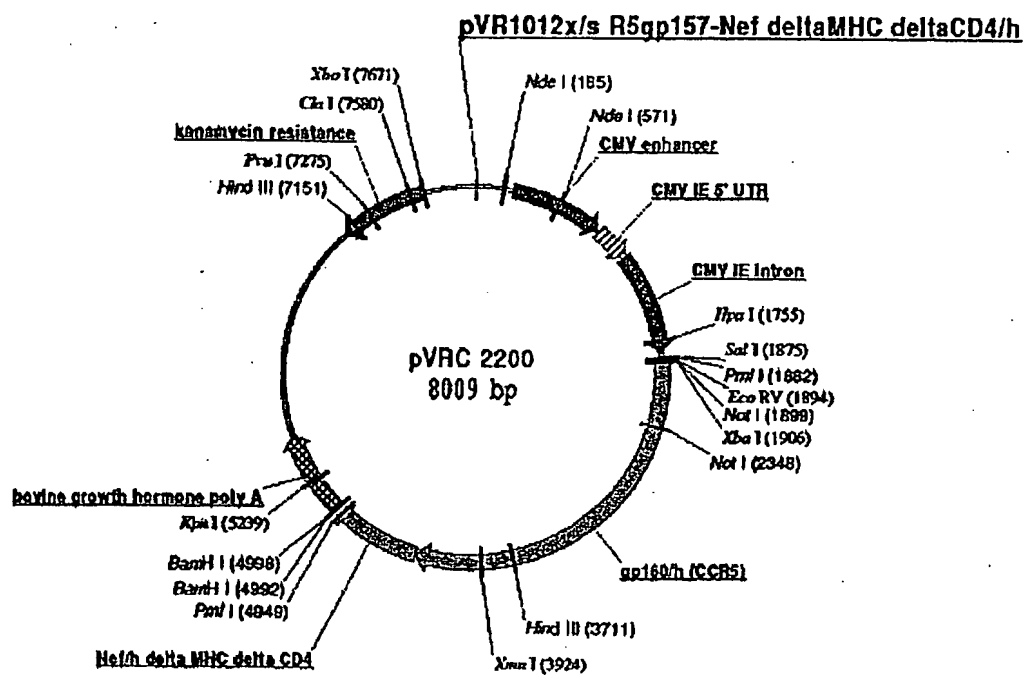


Figure 2

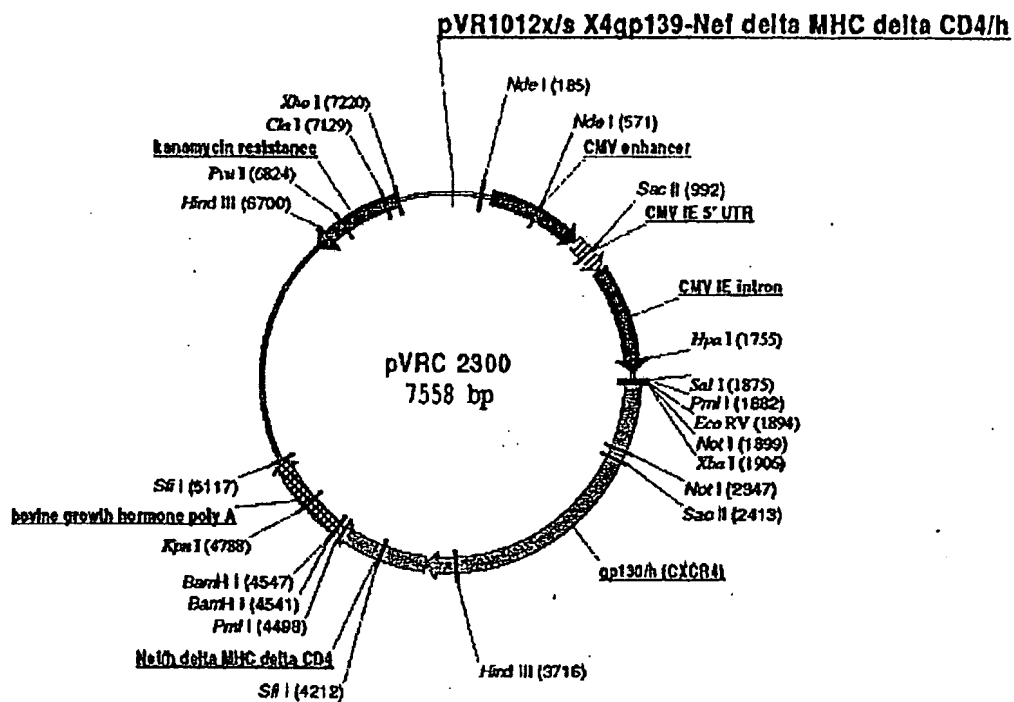


Figure 3

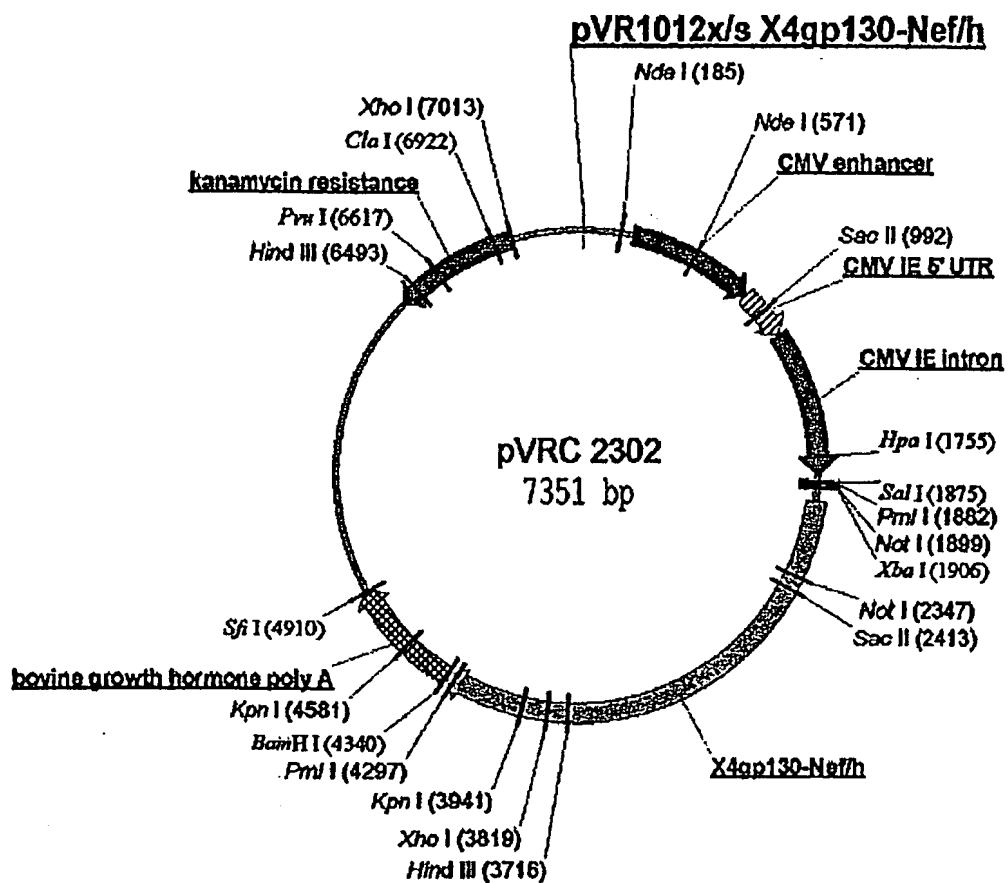


Figure 4

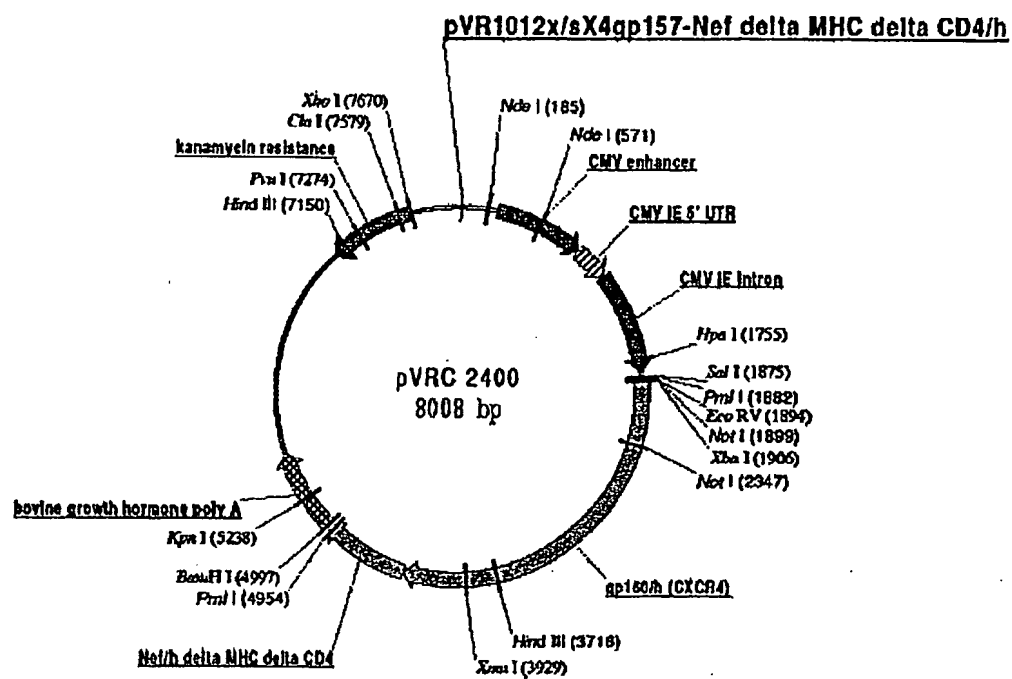


Figure 5

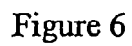


Figure 6

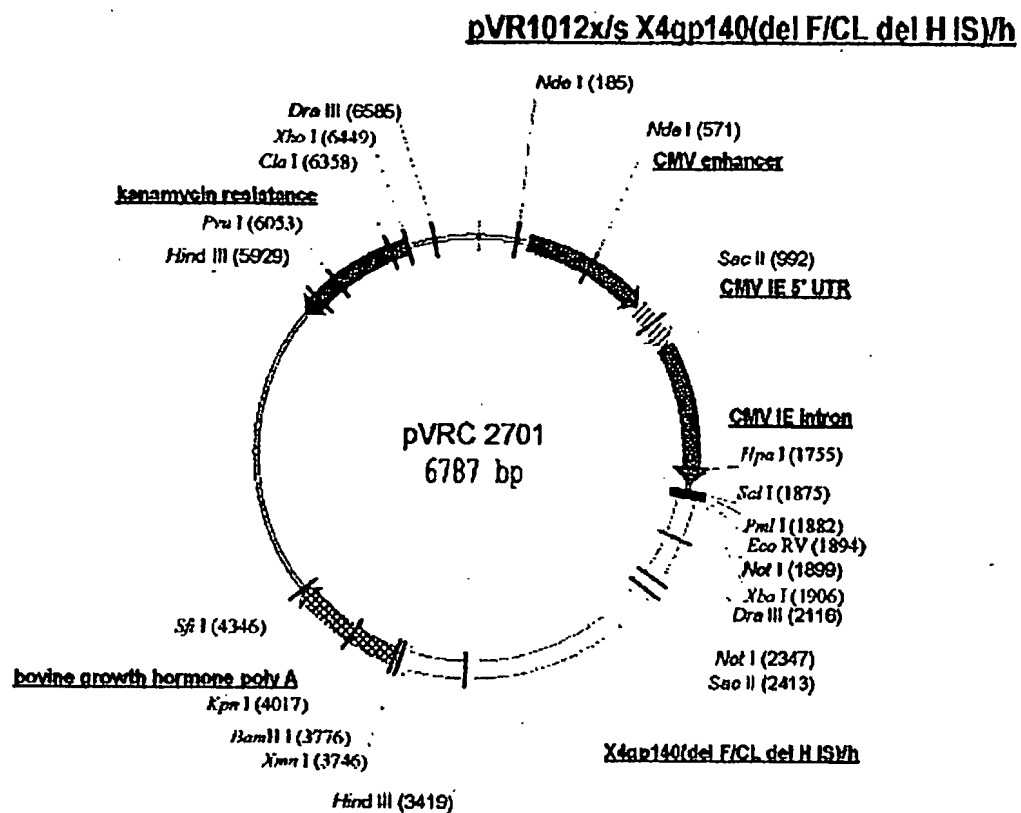


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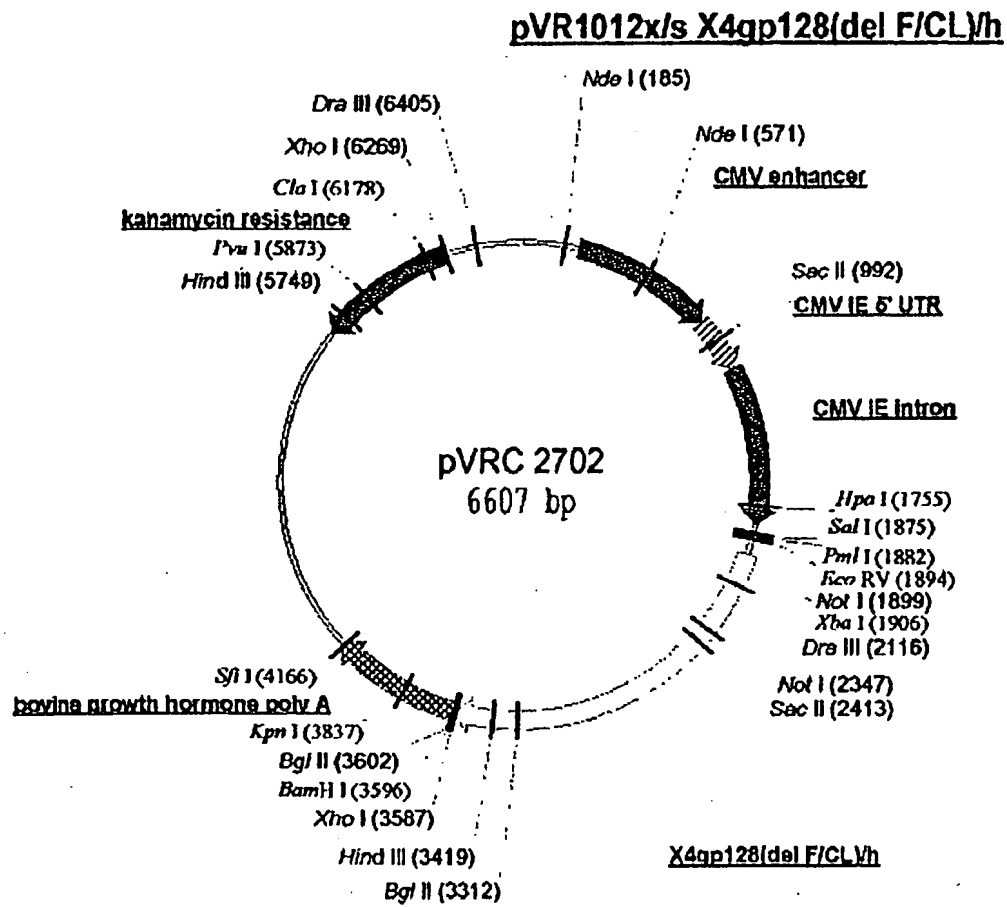


Figure 8

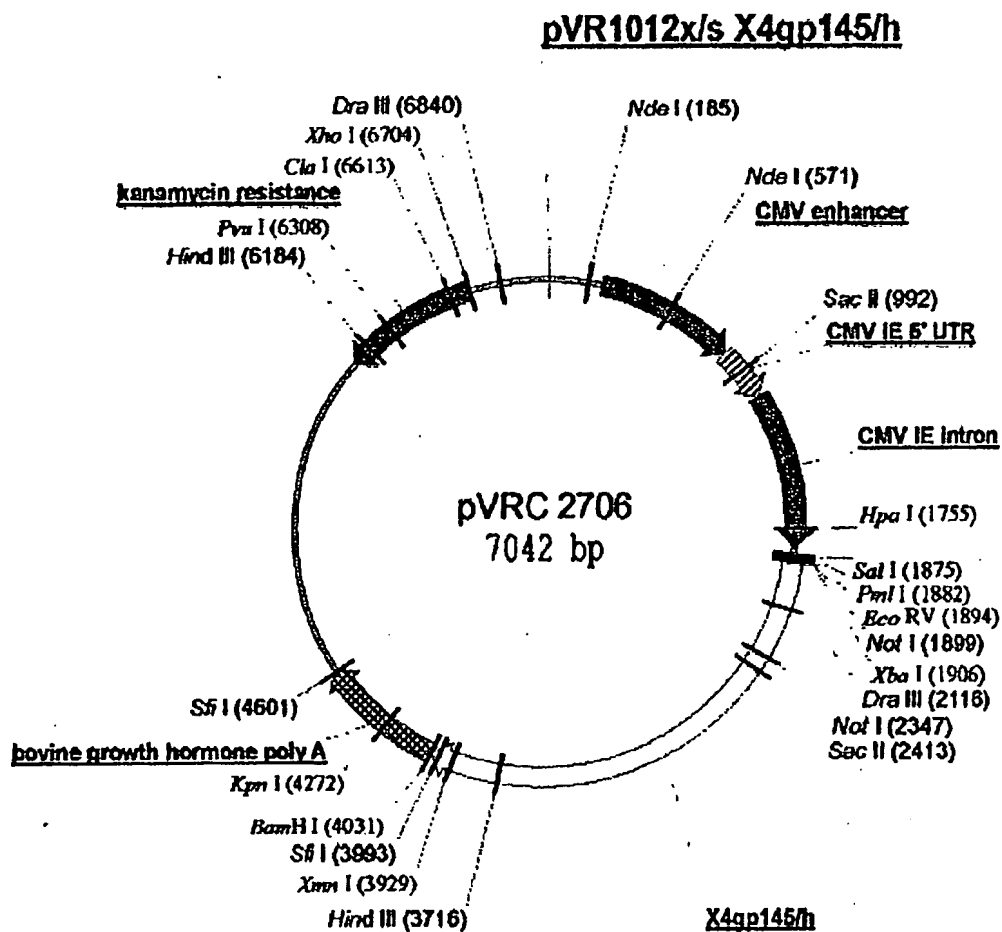


Figure 9

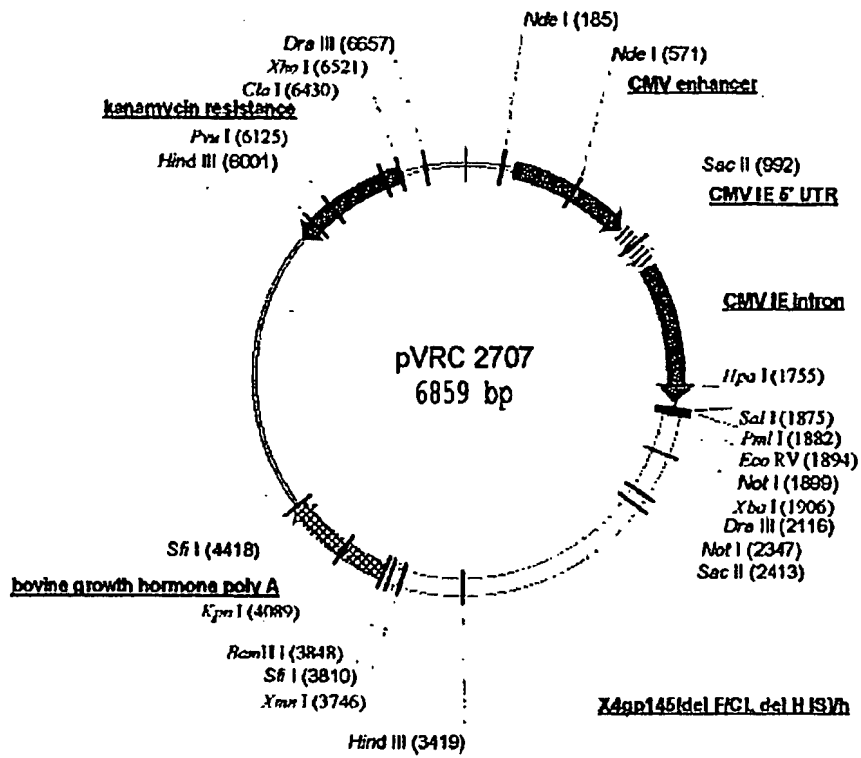
pVR1012x/s X4gp145(del F/CL del HIS)/h

Figure 10

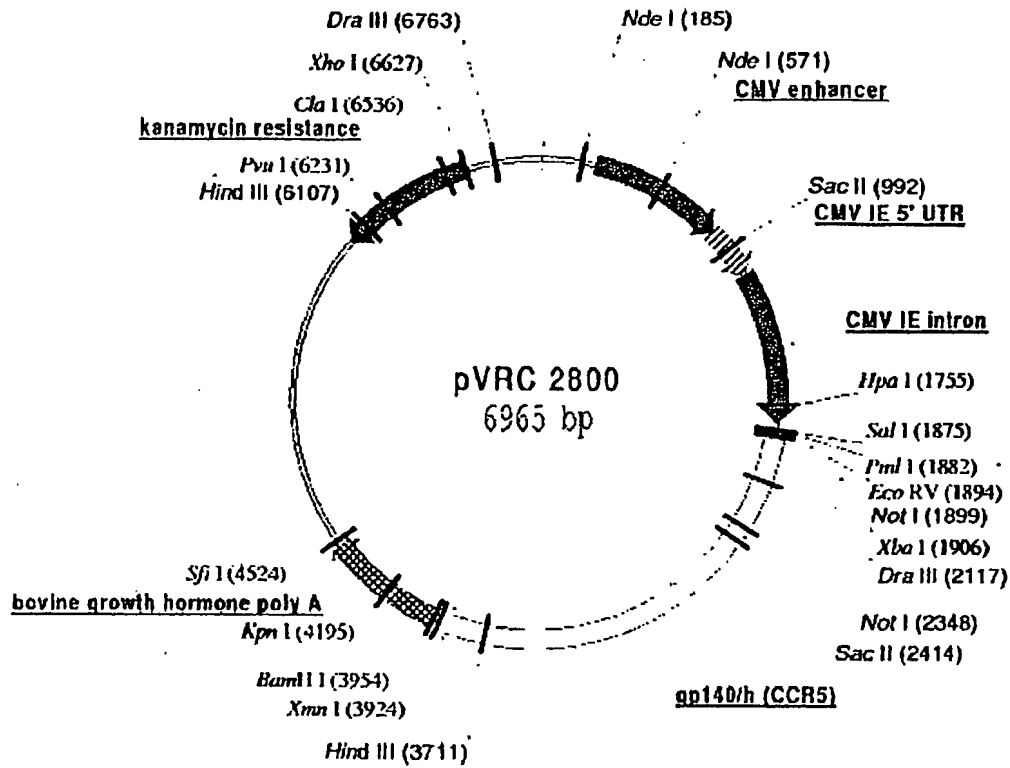
pVR1012x/s R5gp140/h

Figure 11

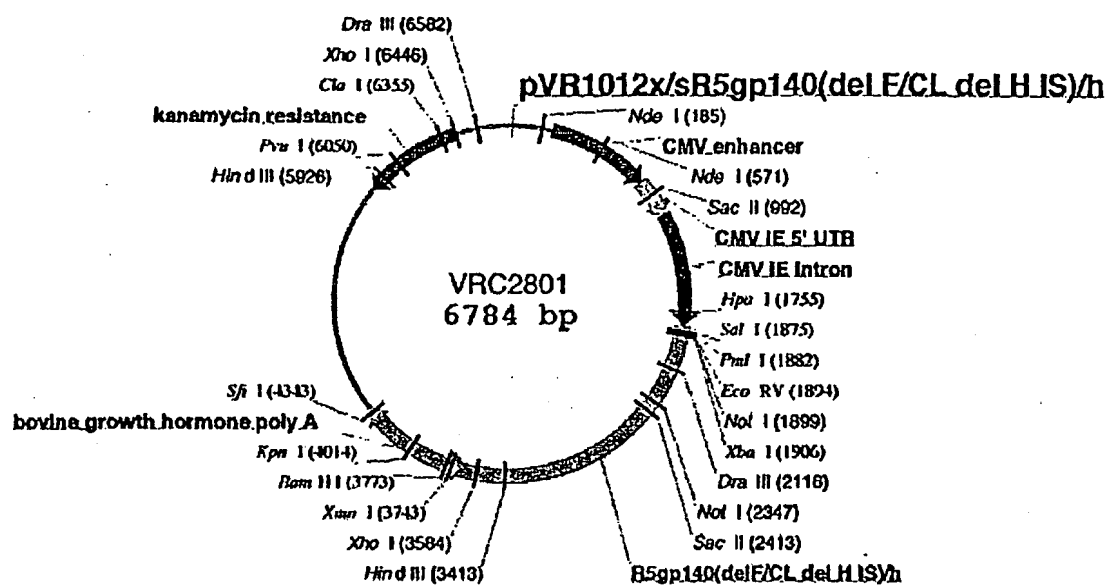


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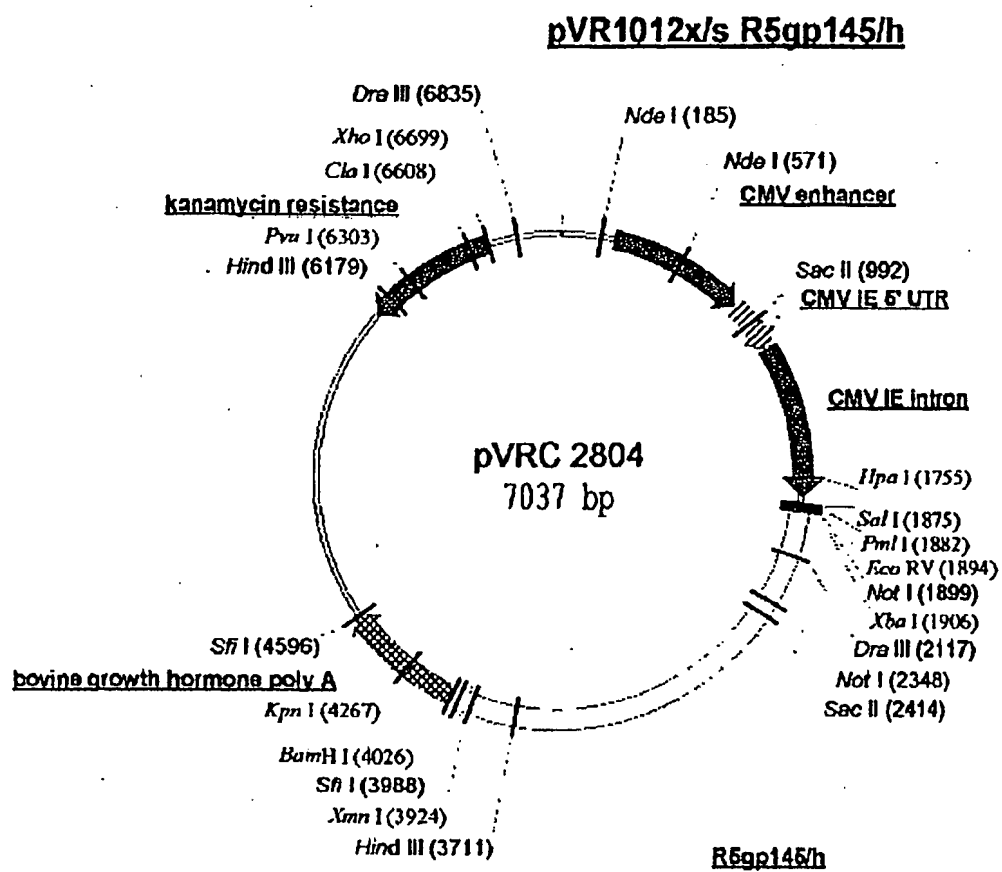


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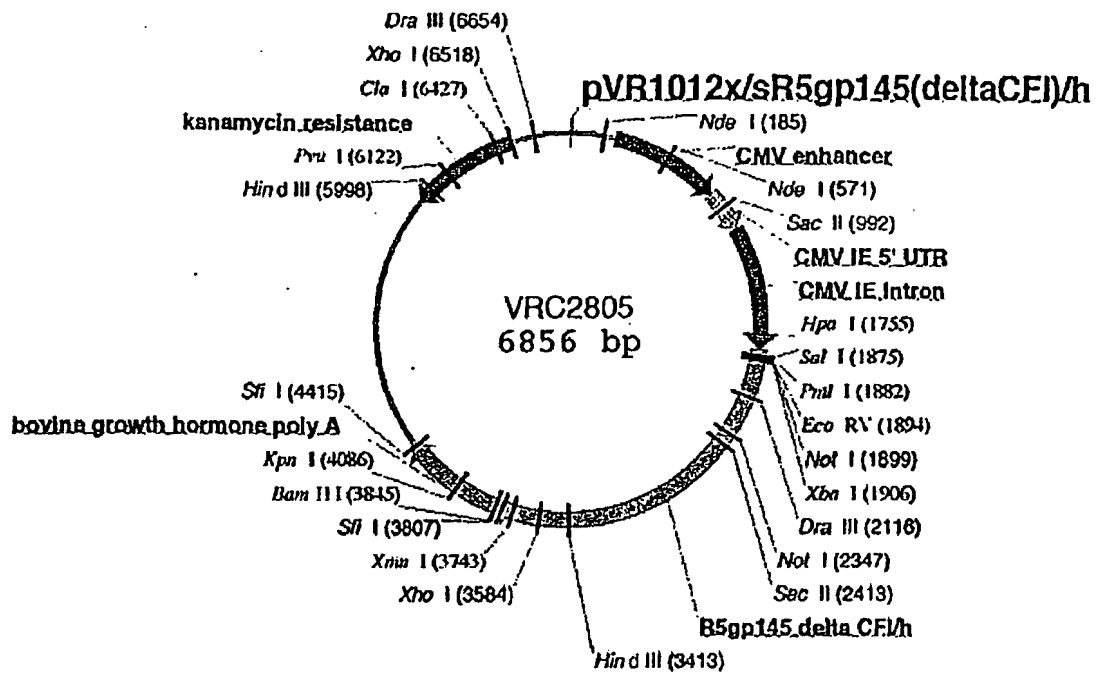


Figure 14

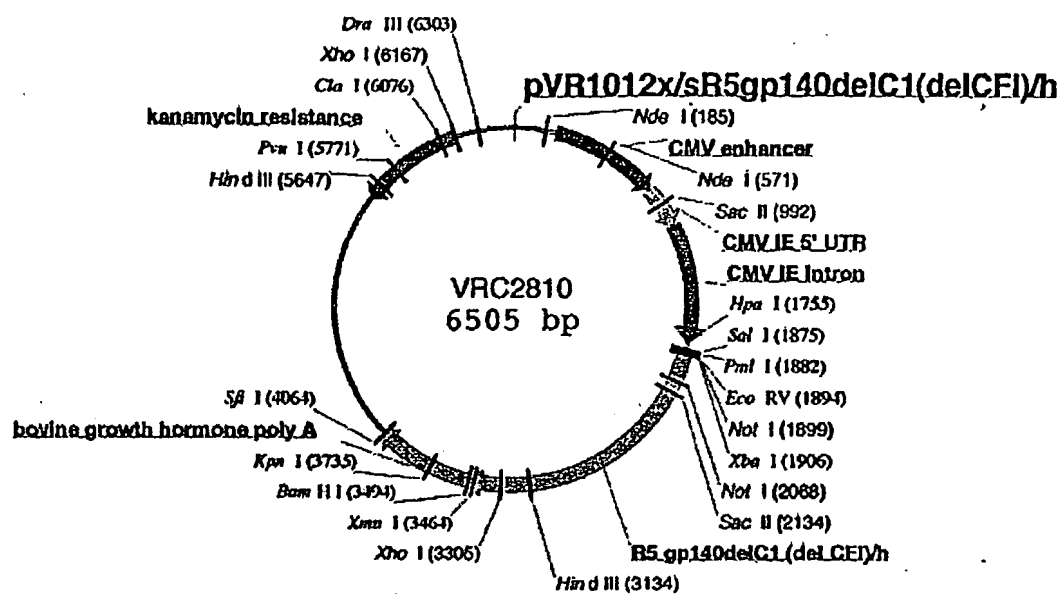


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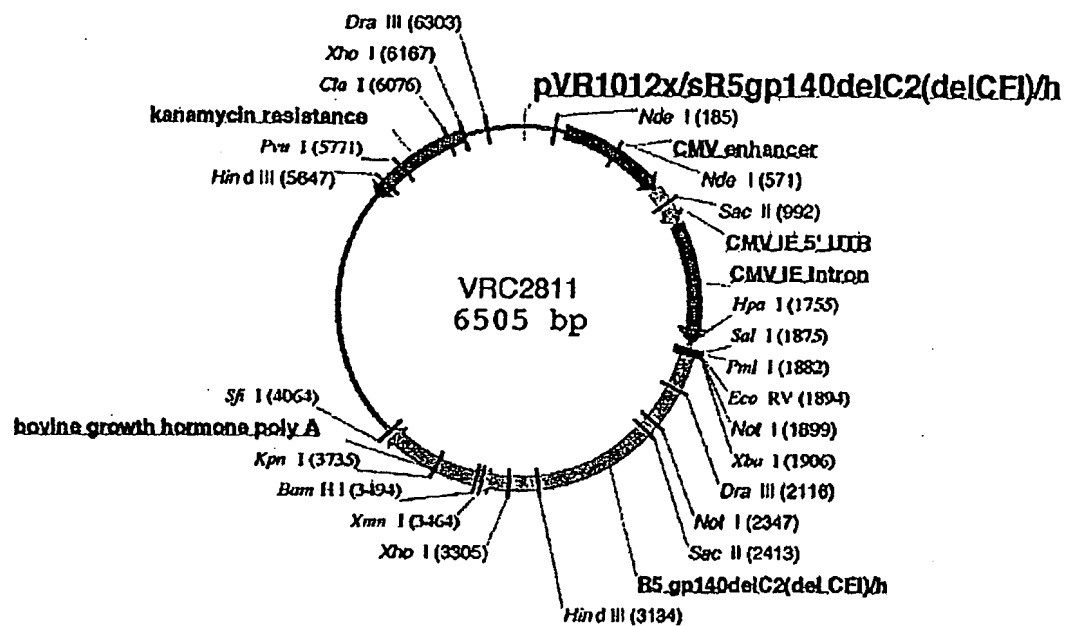


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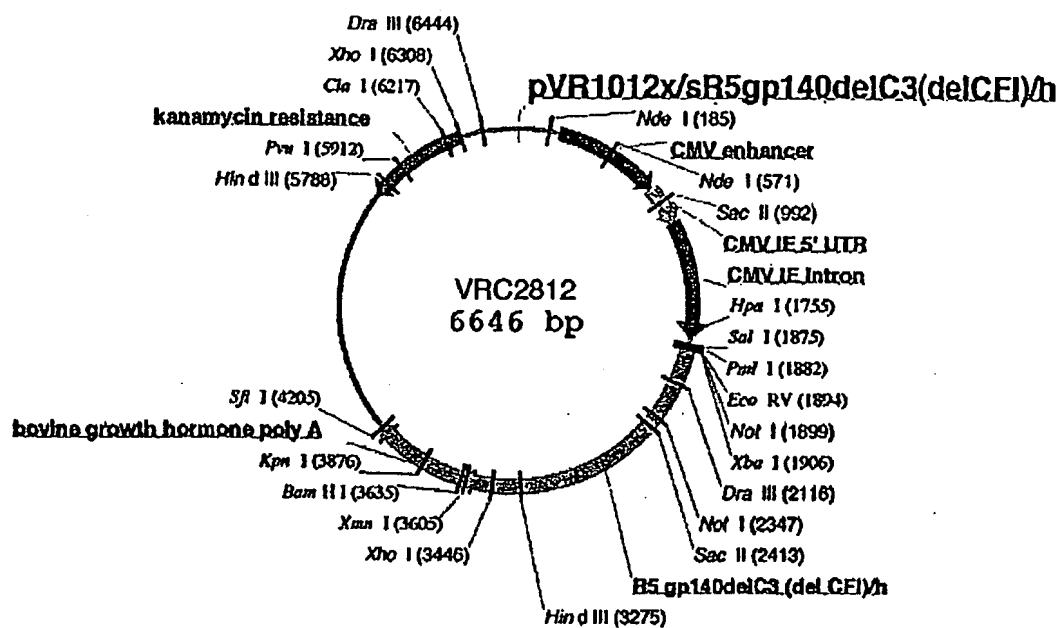


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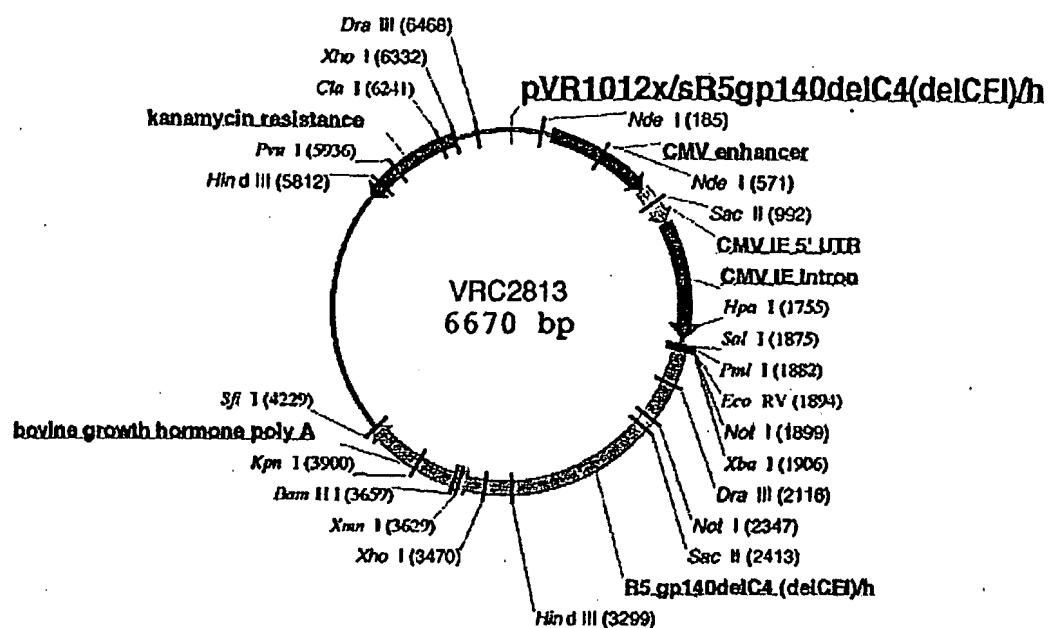


Figure 18

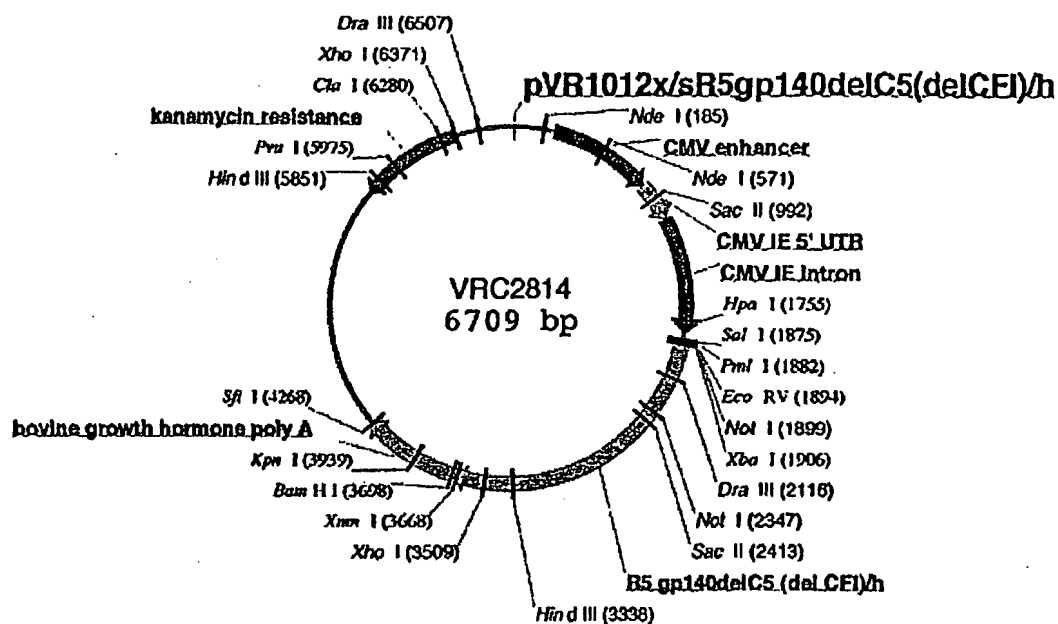


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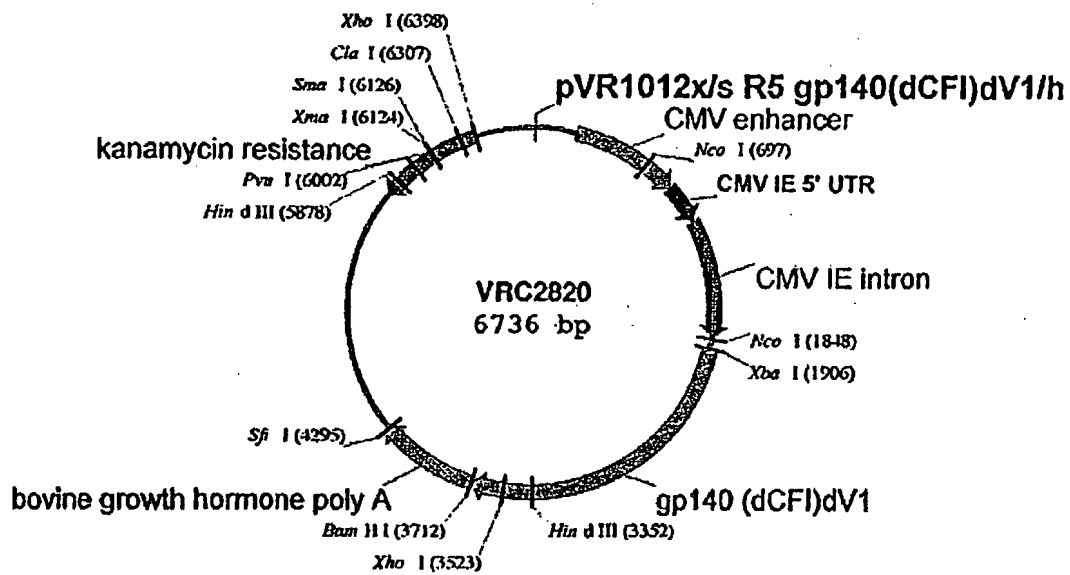


Figure 20

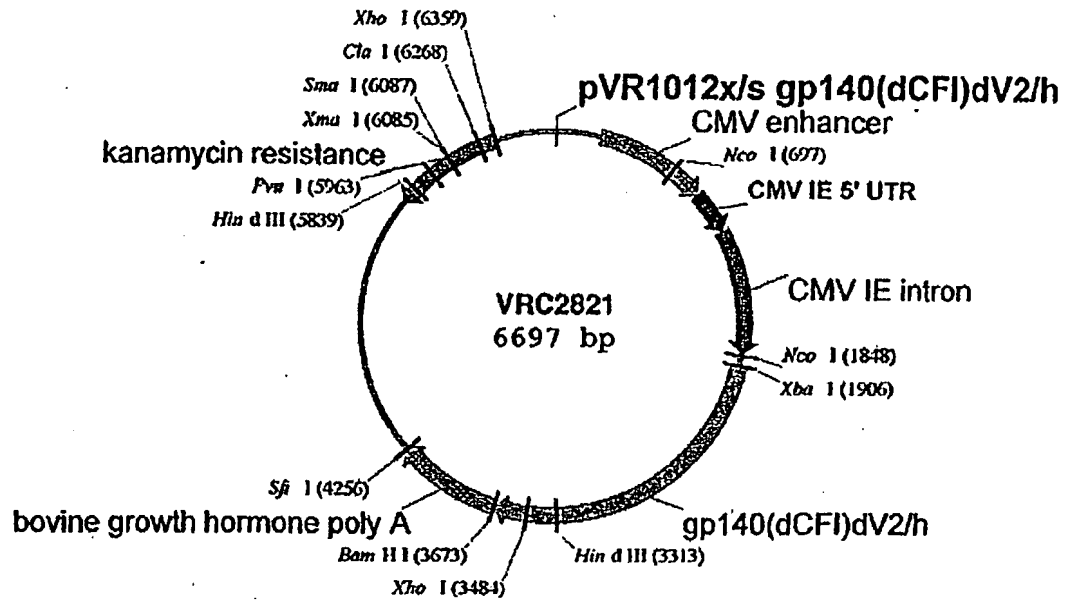


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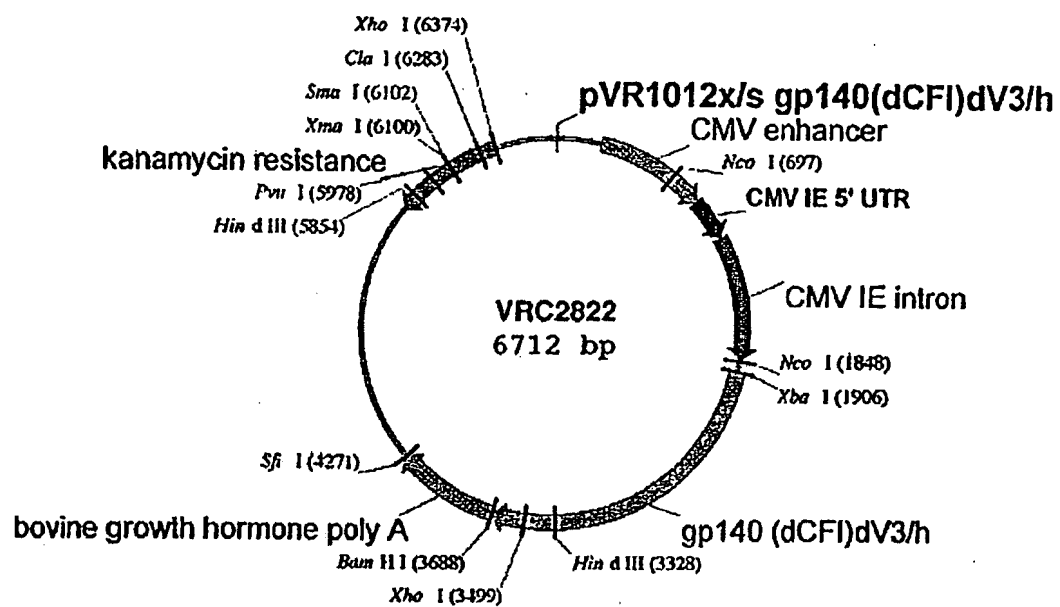


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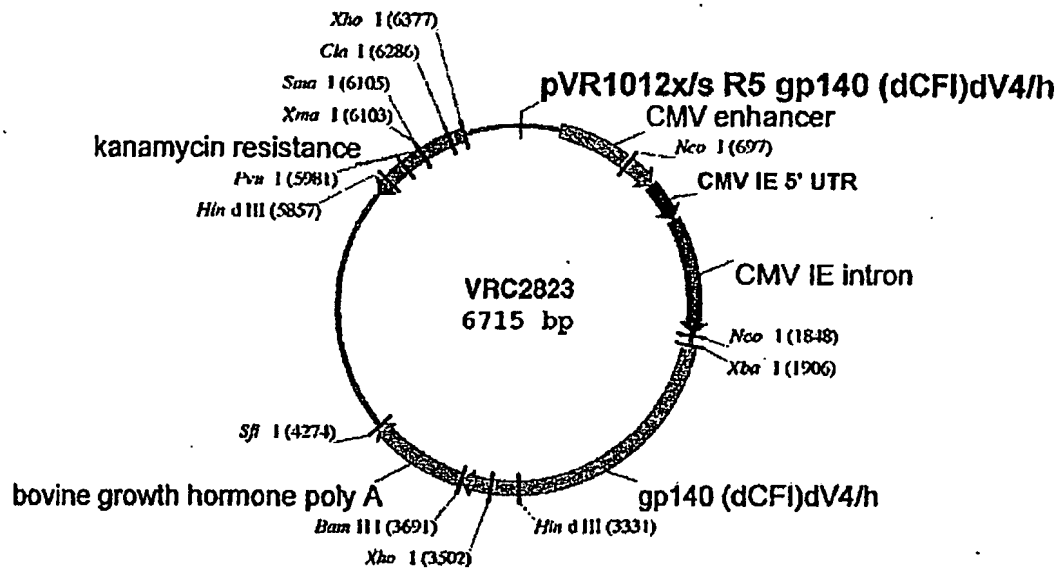


Figure 23

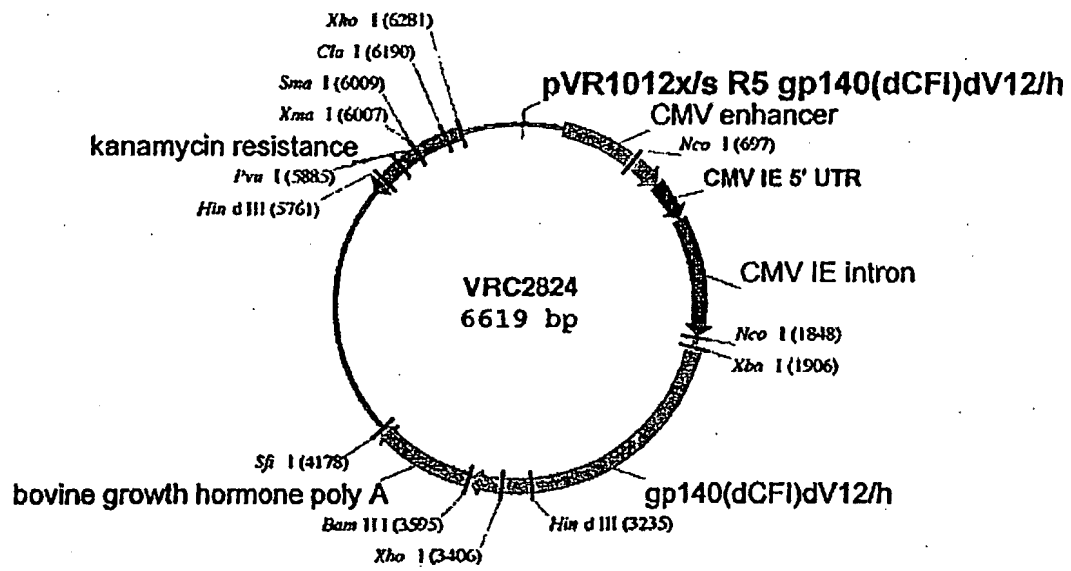


Figure 24

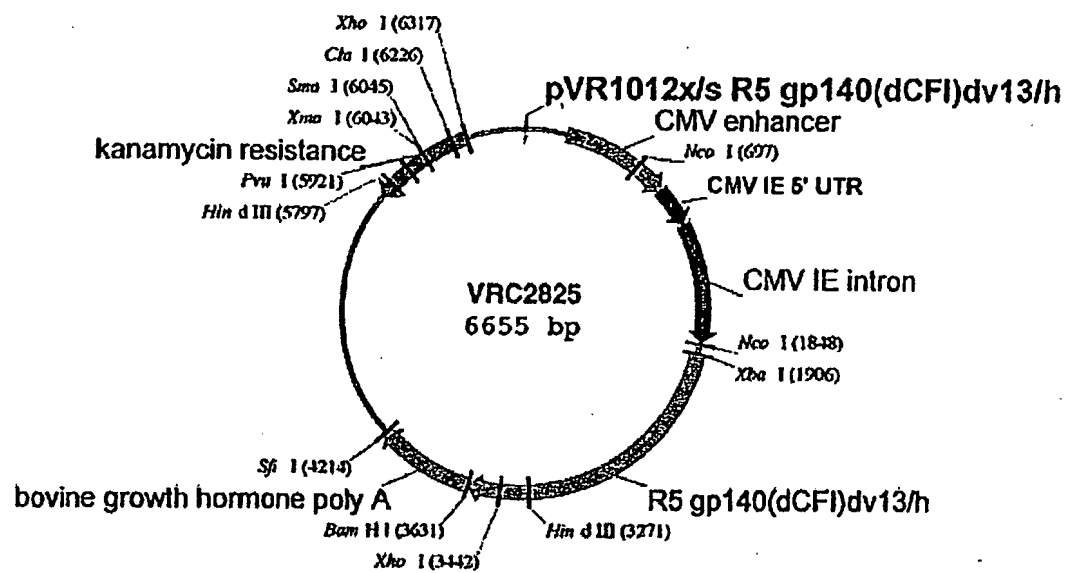


Figure 25

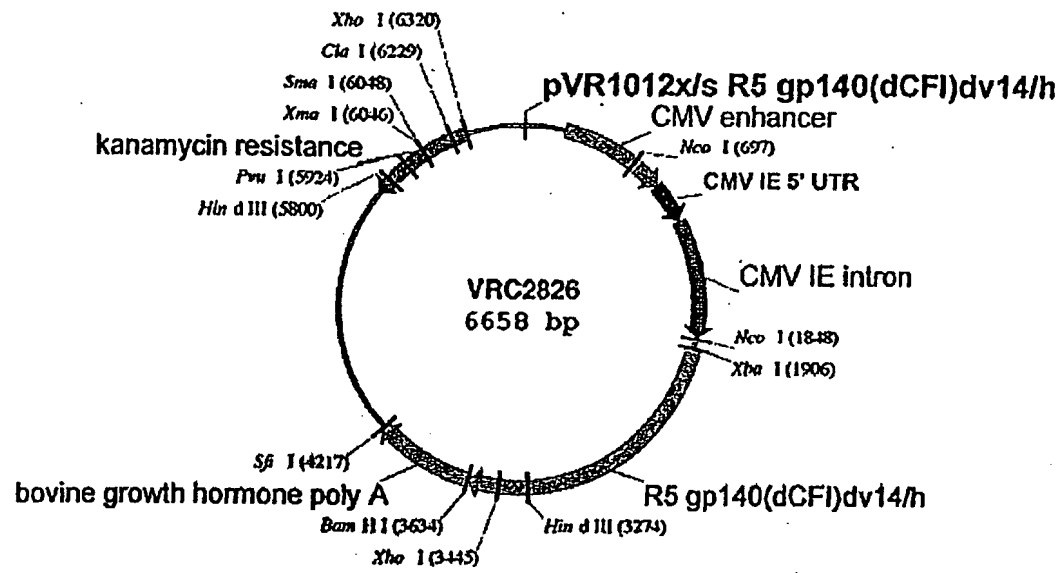


Figure 26

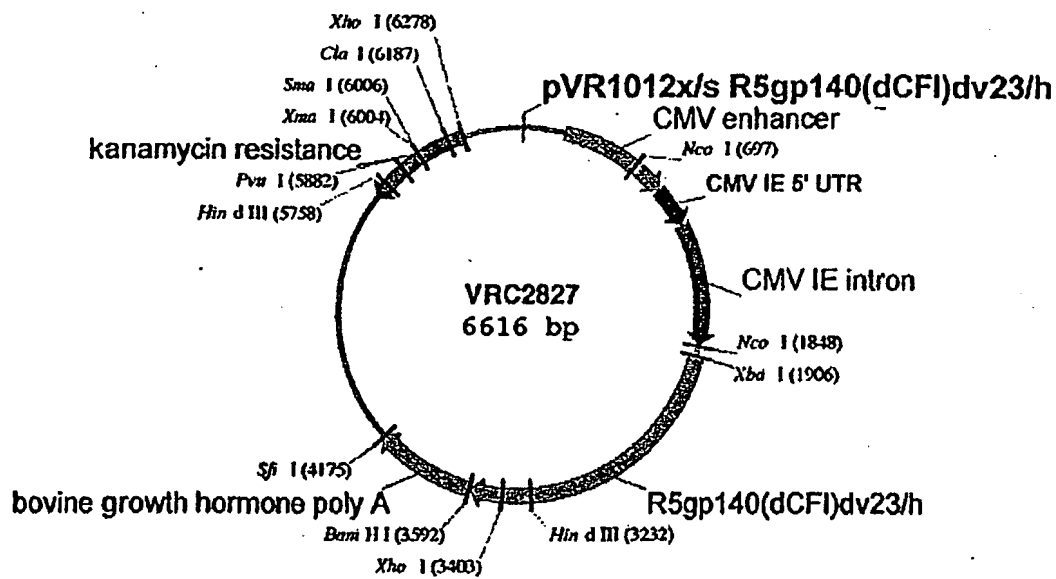


Figure 27

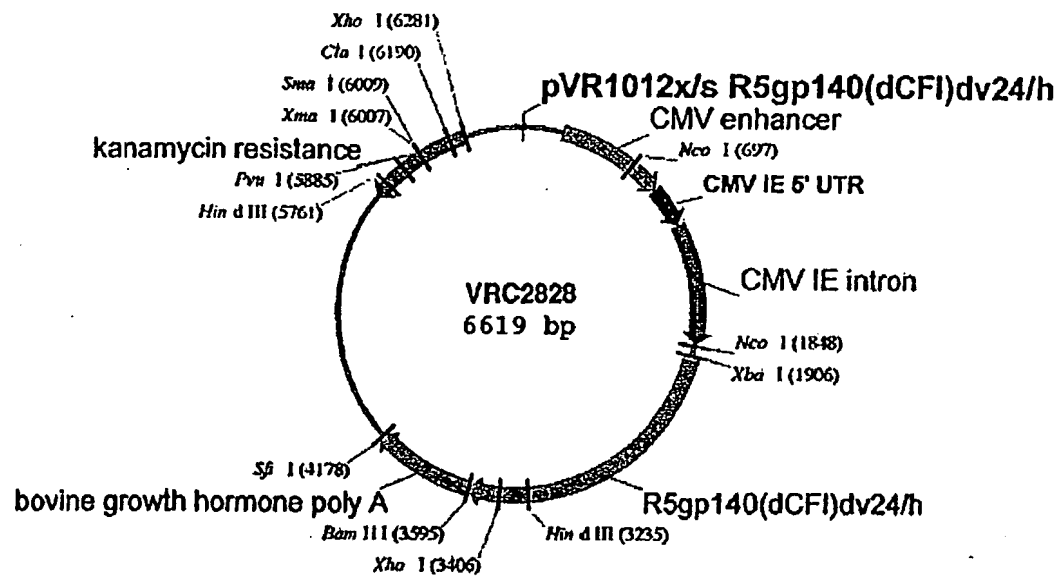


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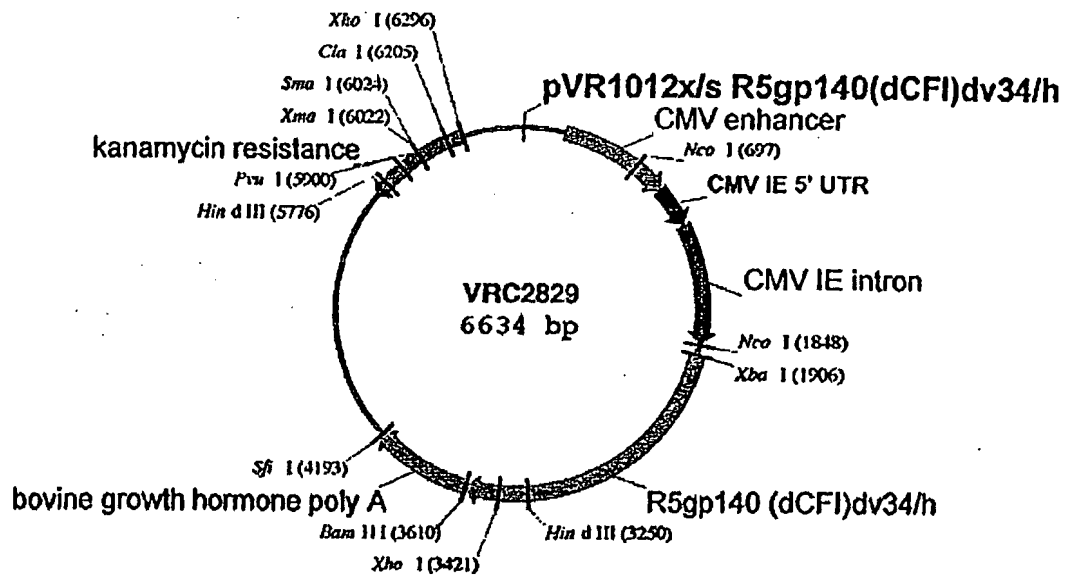


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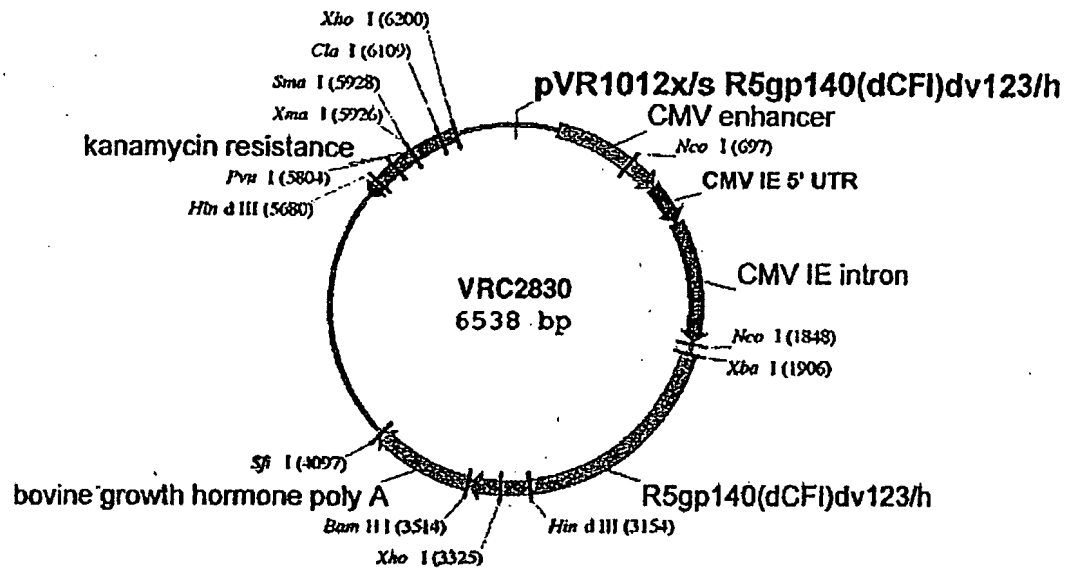


Figure 30

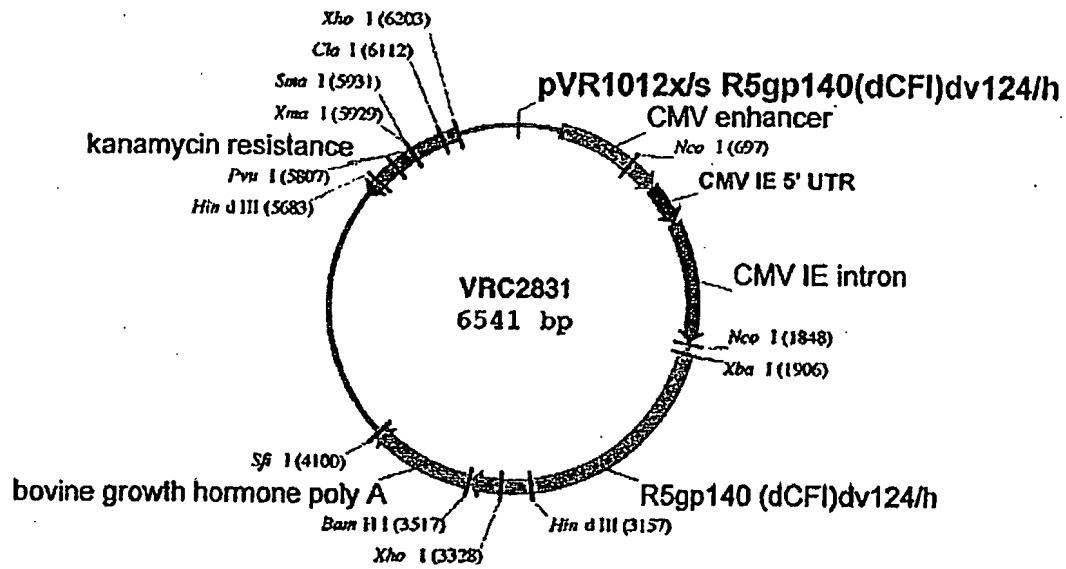


Figure 31

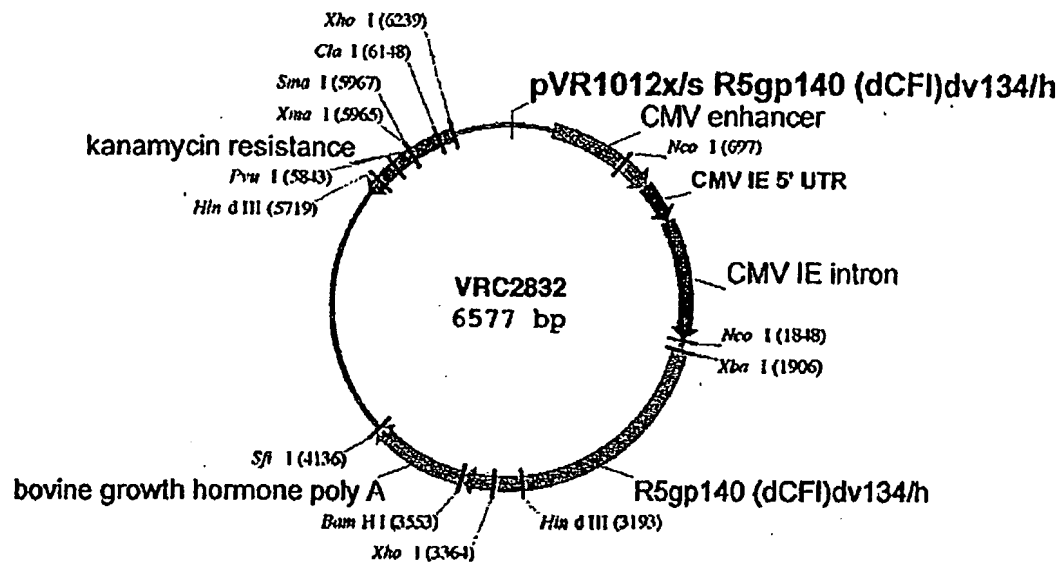


Figure 32

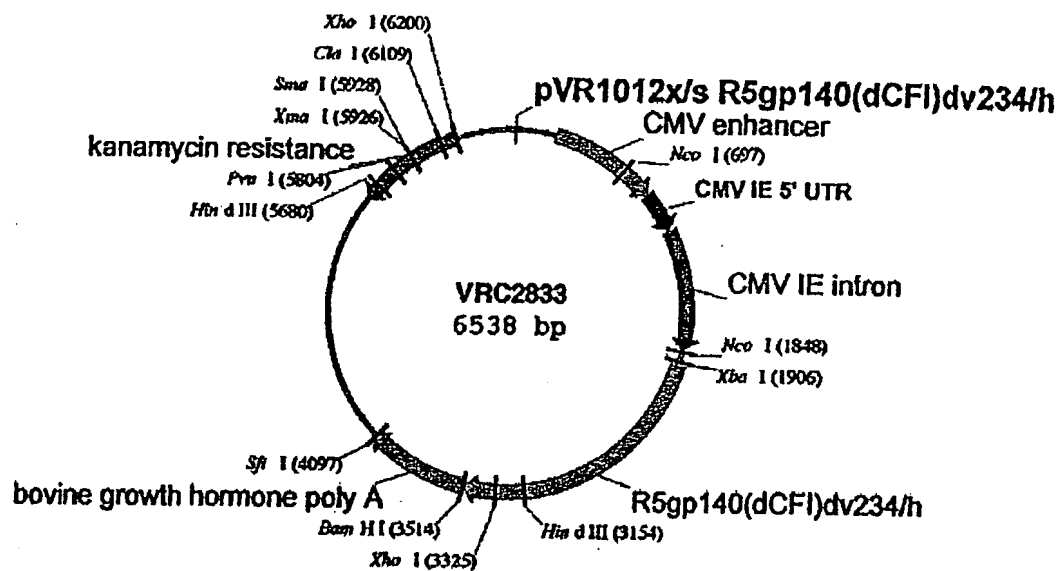


Figure 33

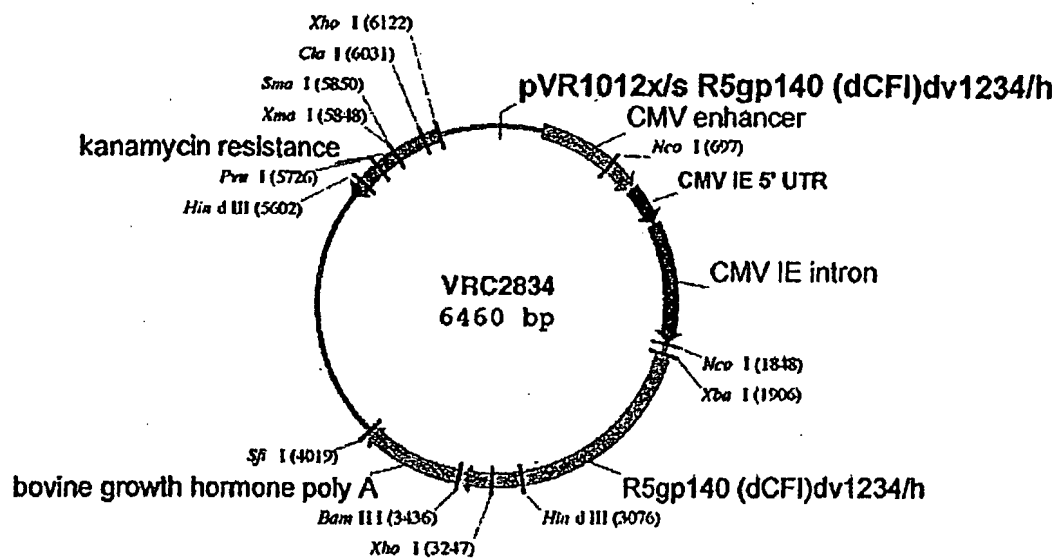


Figure 34

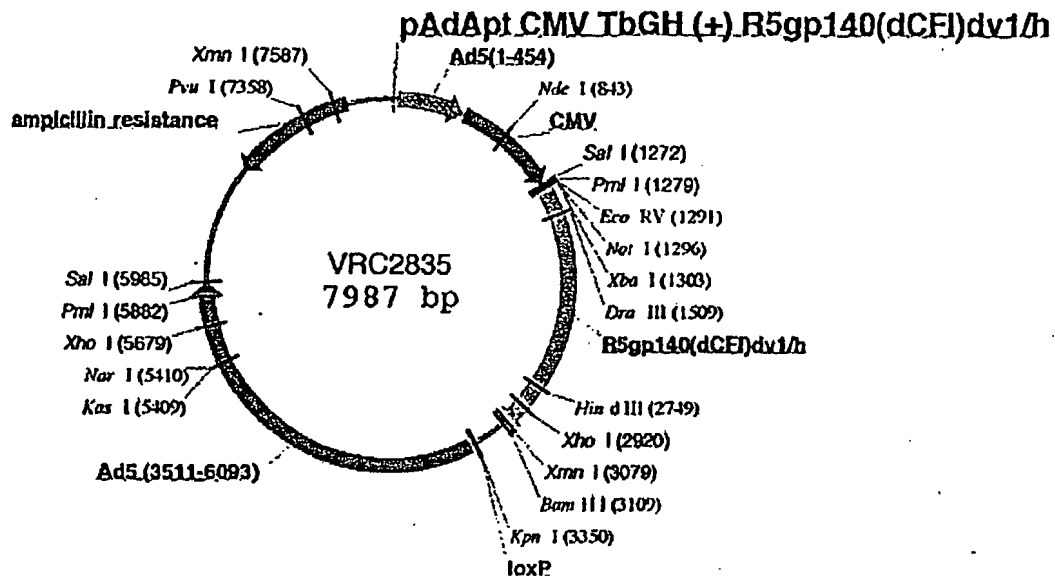


Figure 35

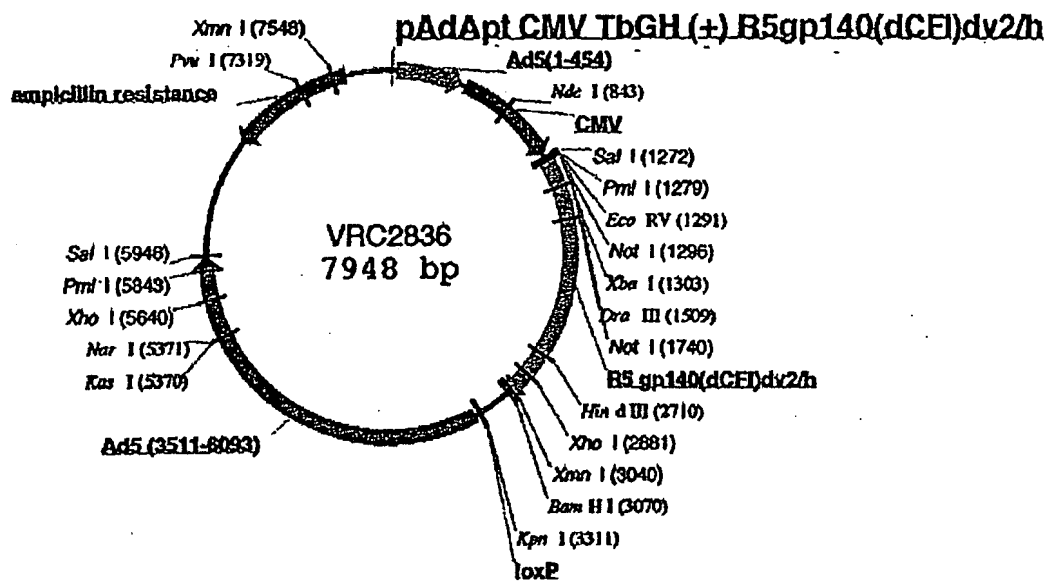


Figure 36

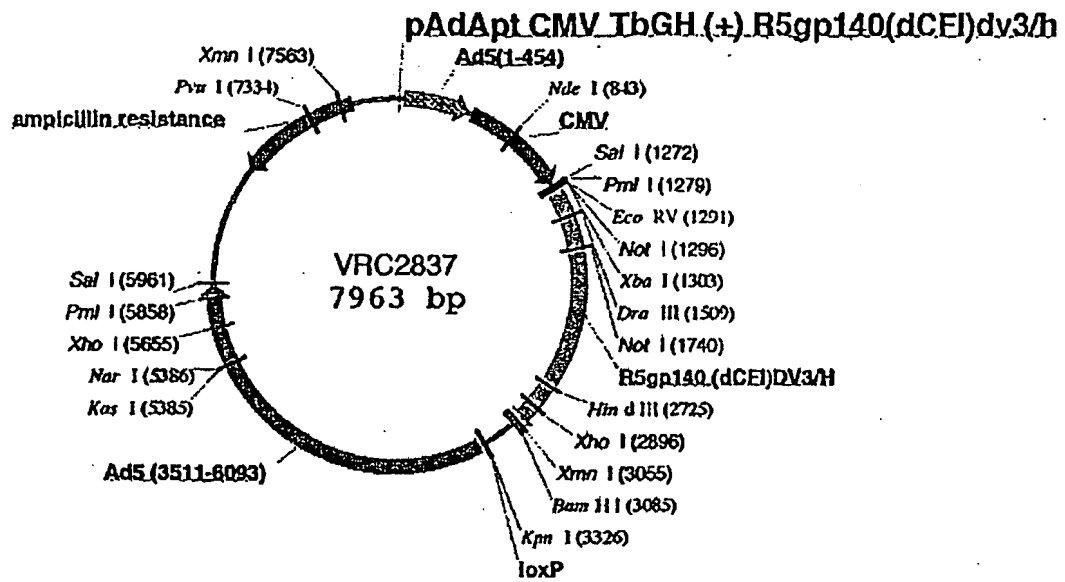


Figure 37

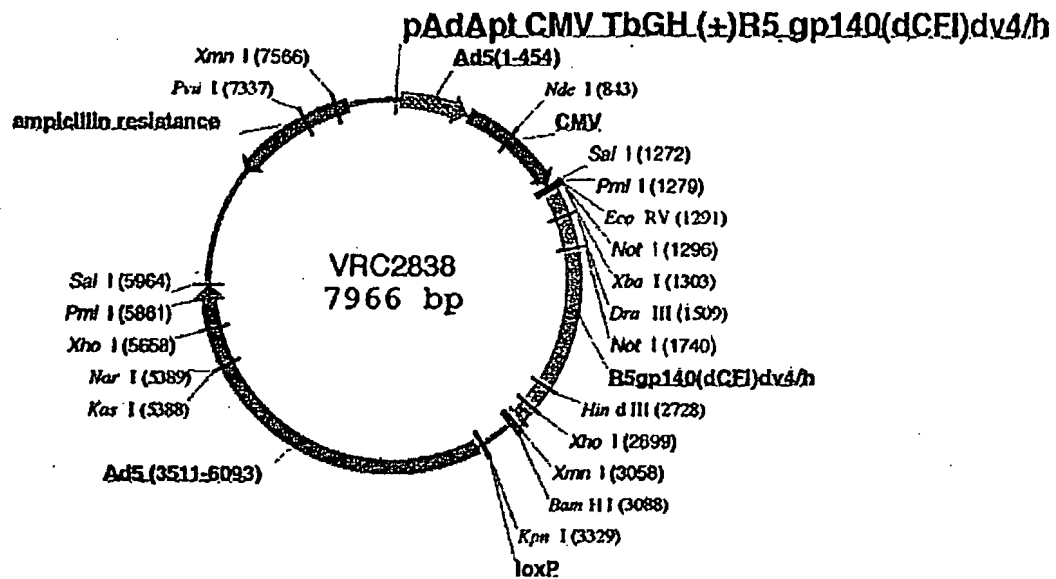


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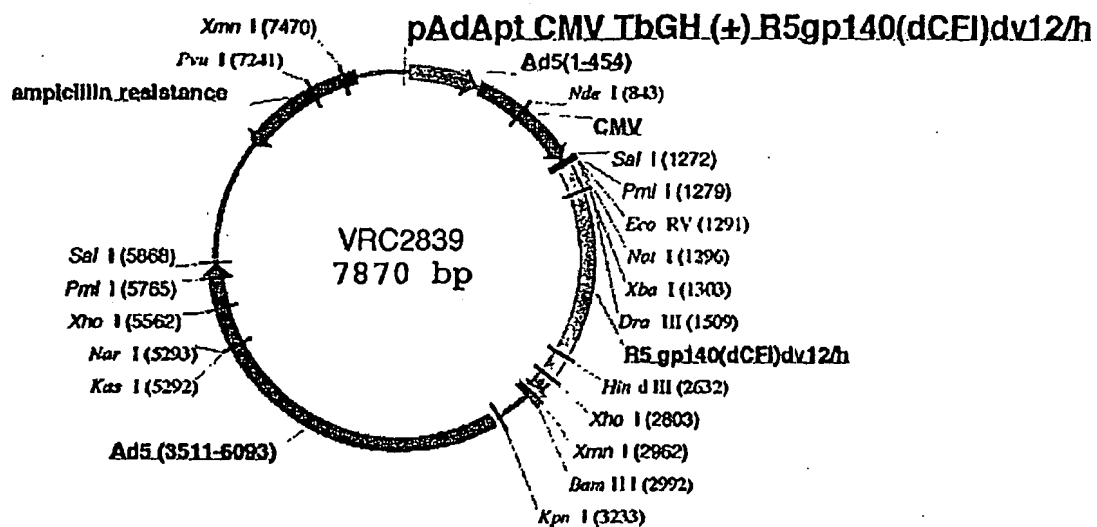


Figure 39

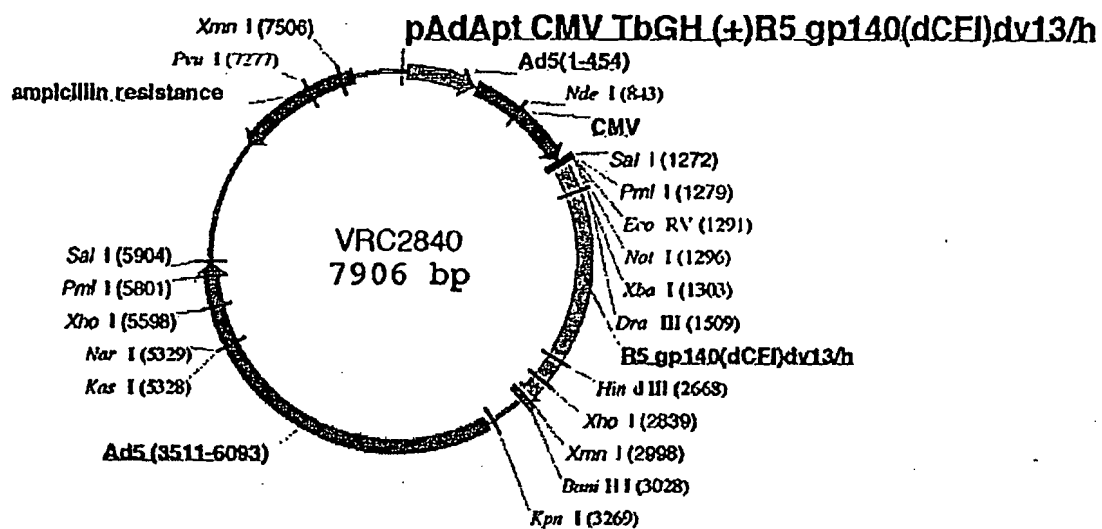


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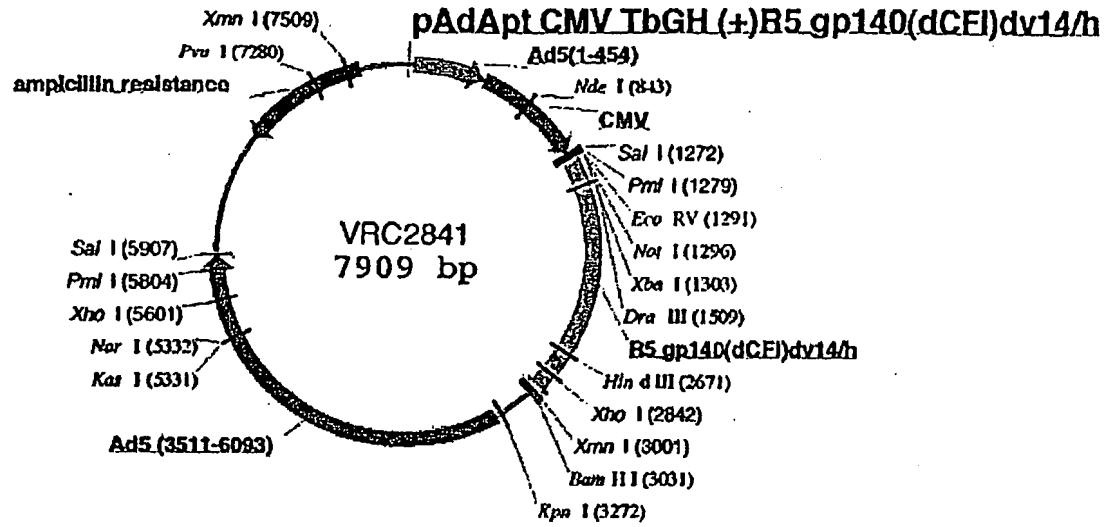


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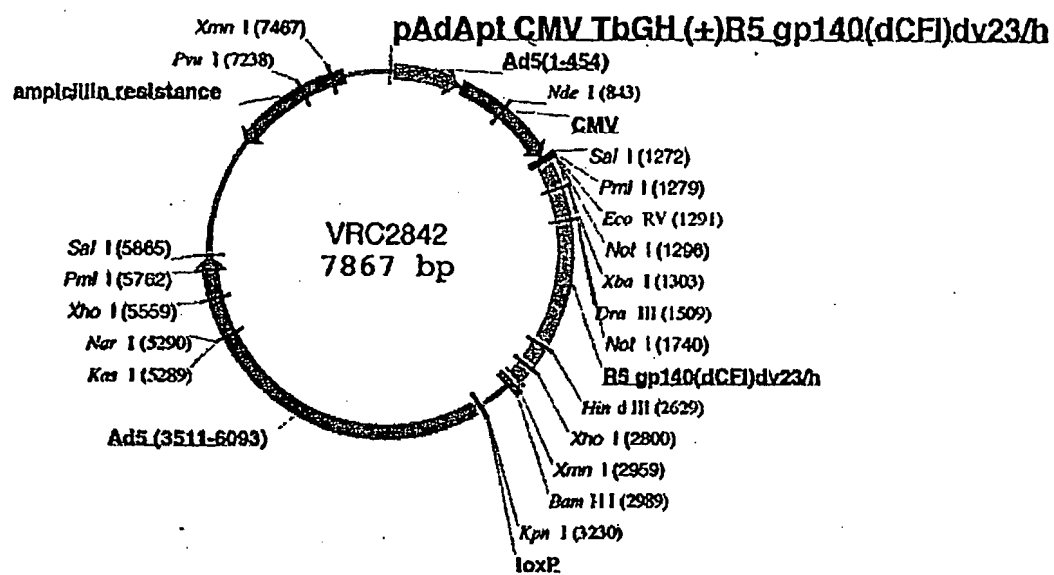


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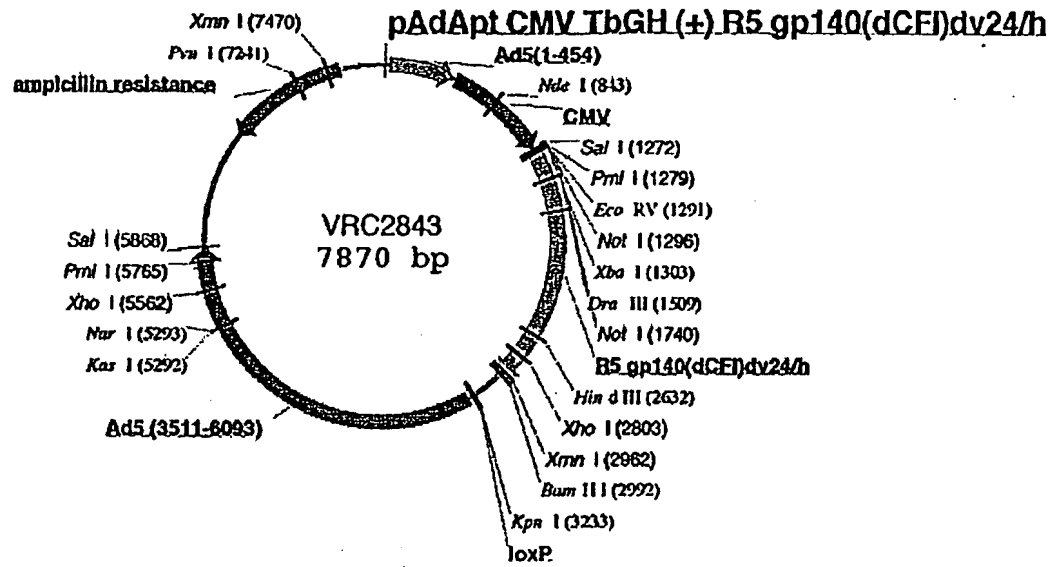


Figure 43

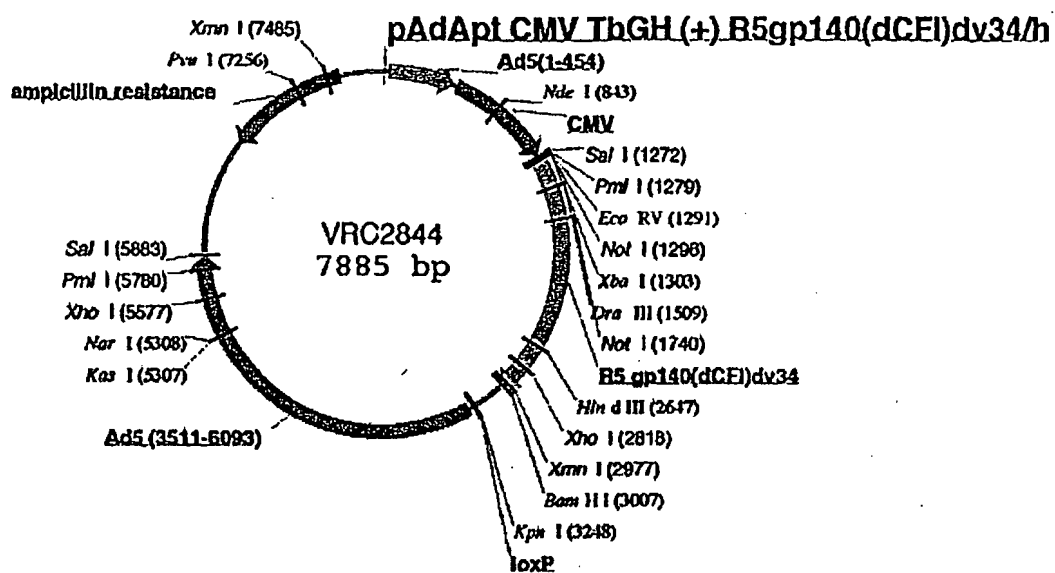


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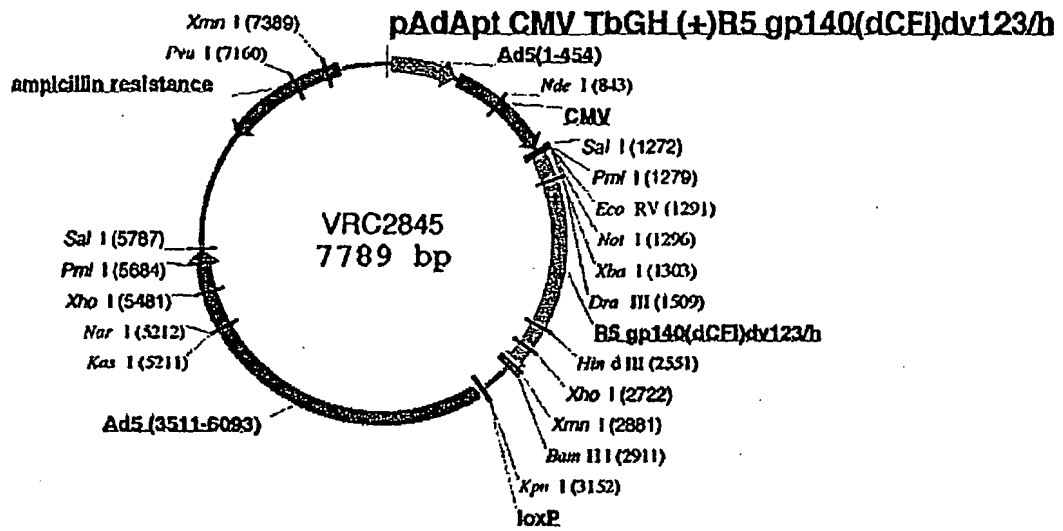


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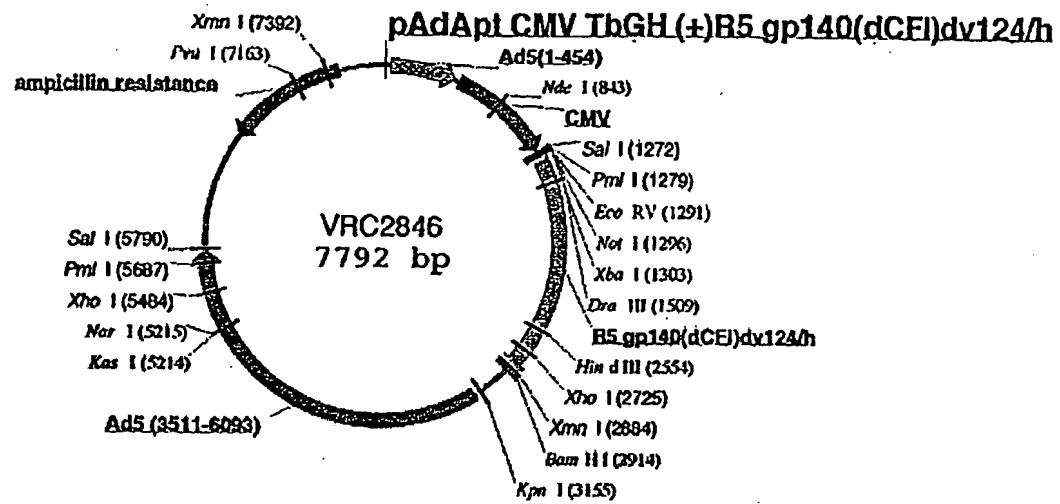


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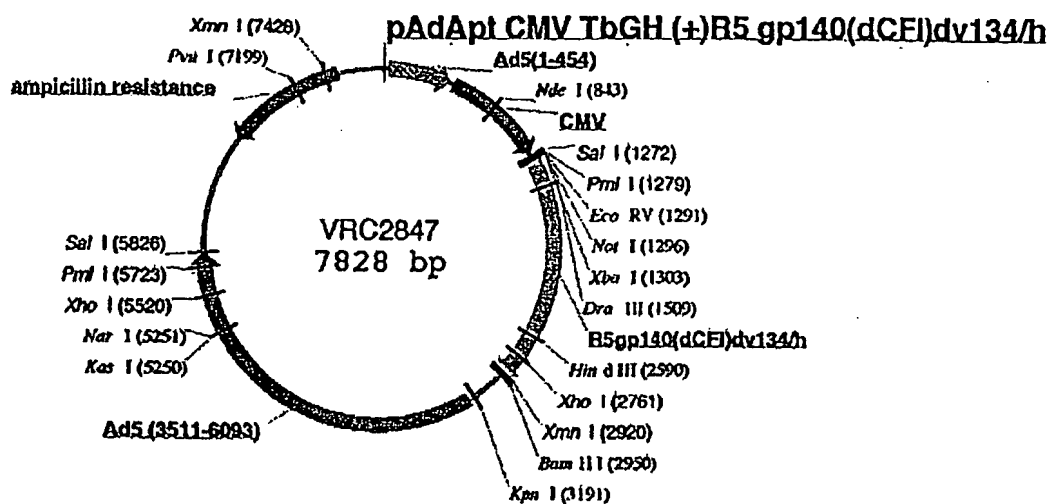


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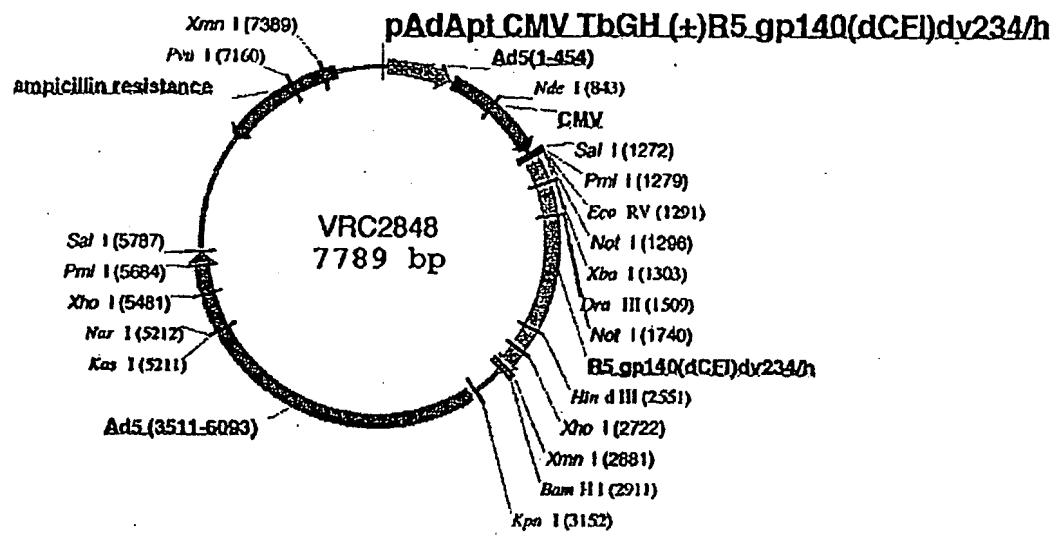


Figure 48

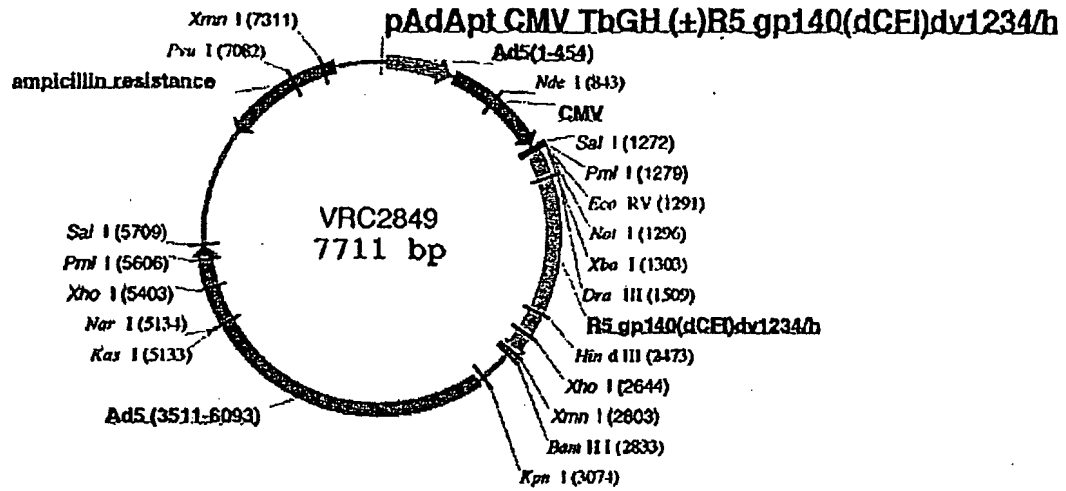


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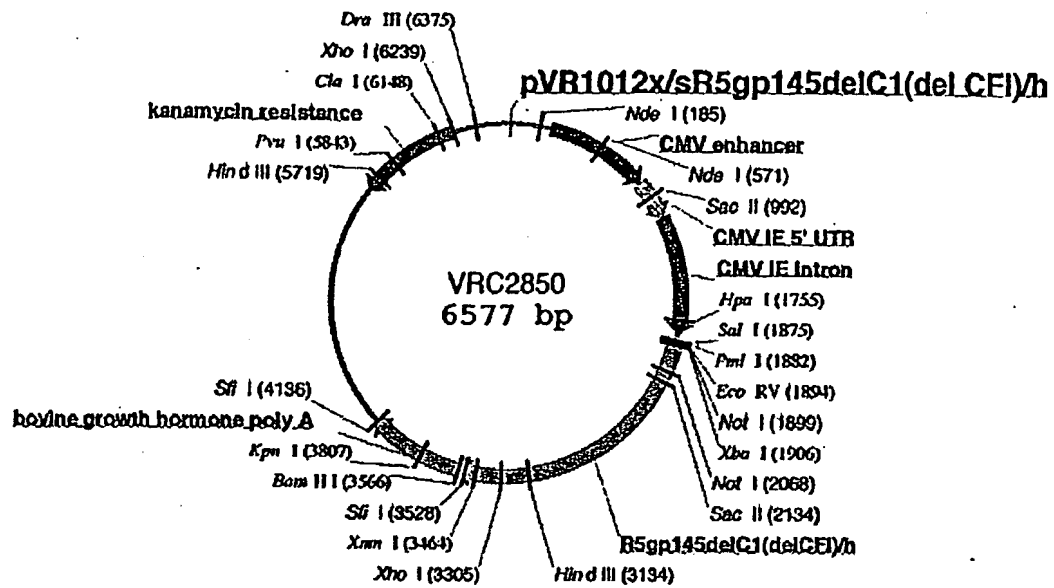


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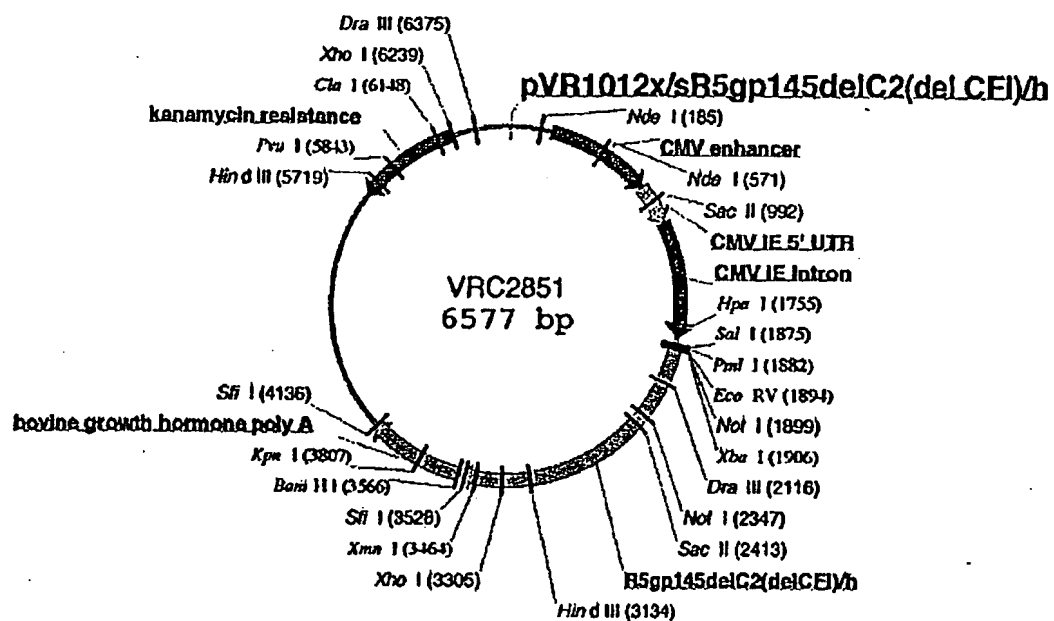


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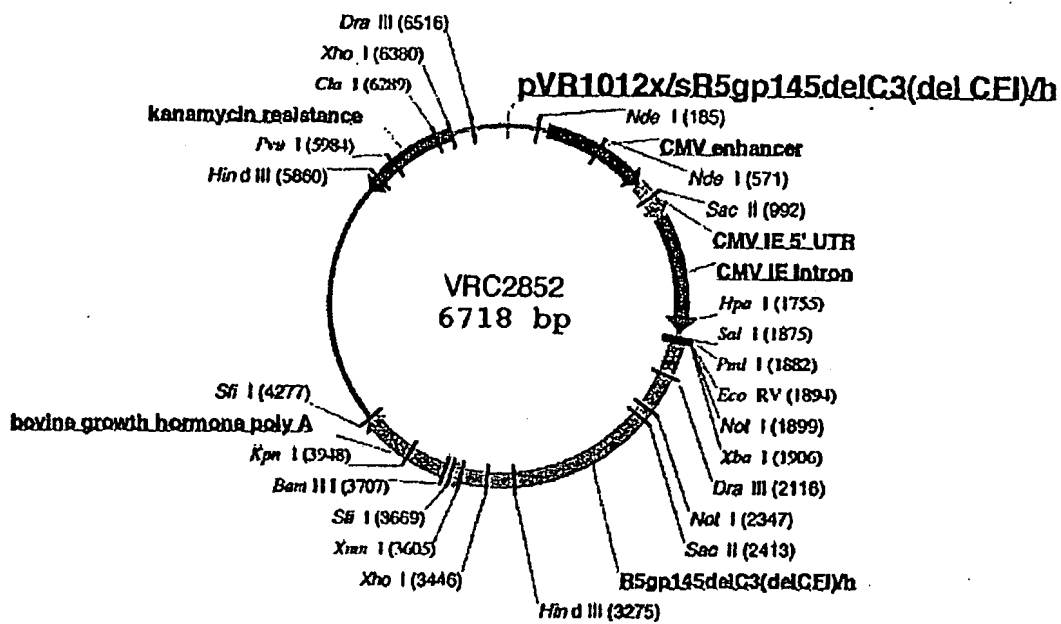


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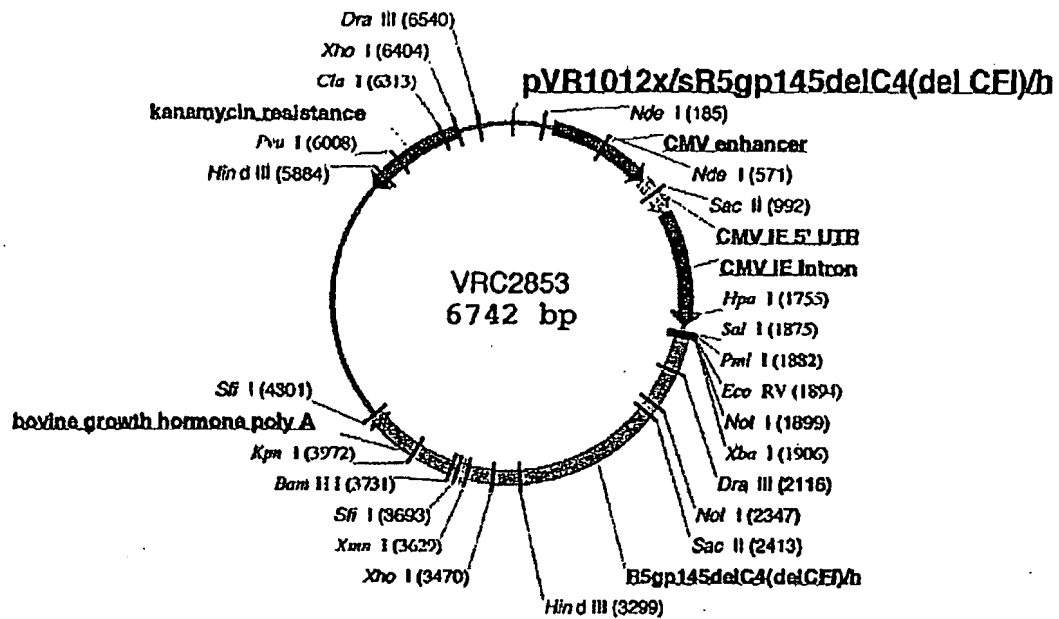


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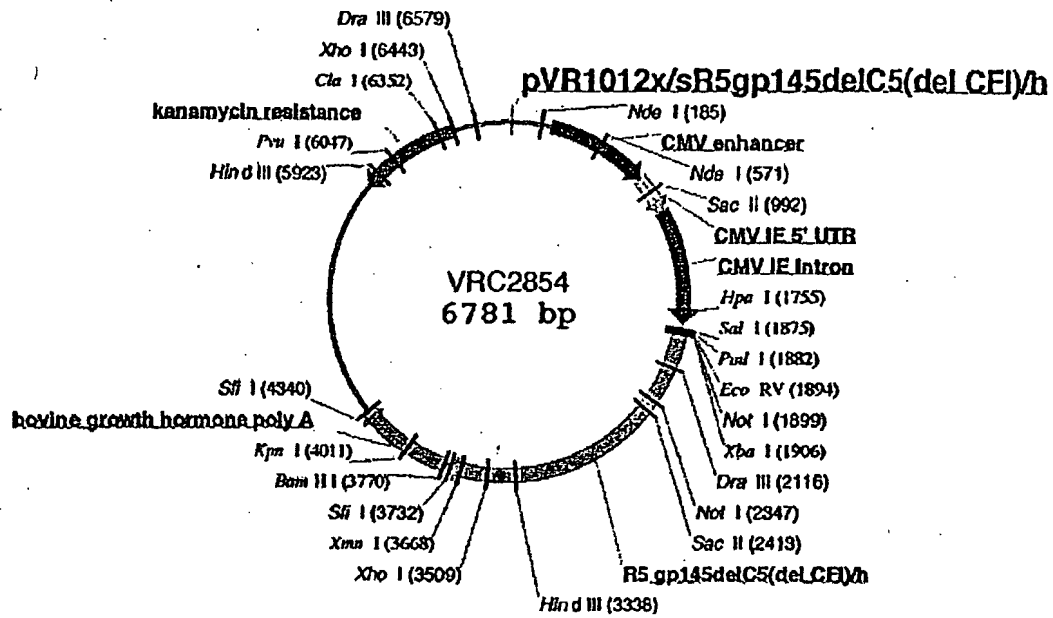


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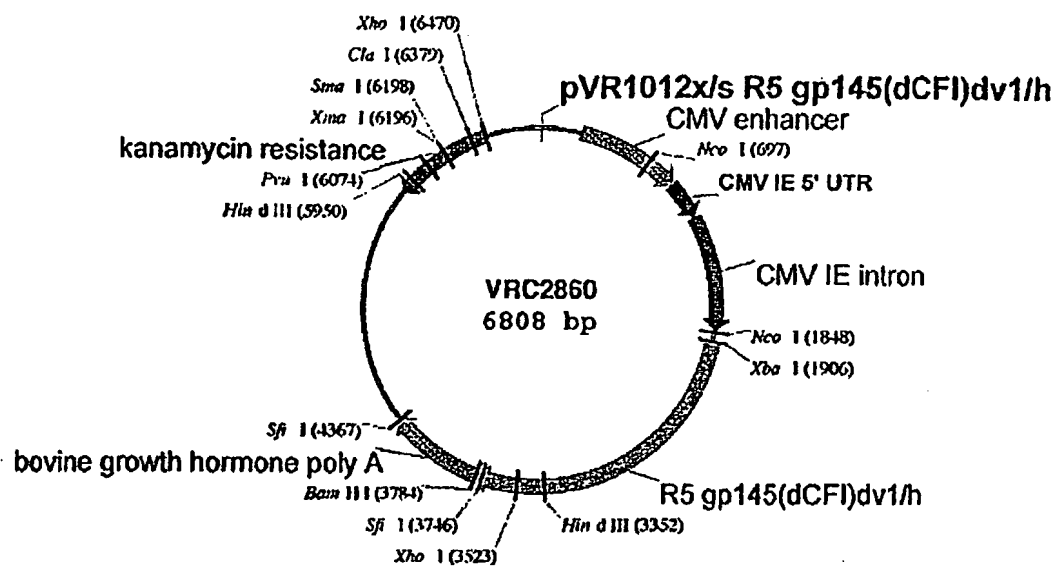


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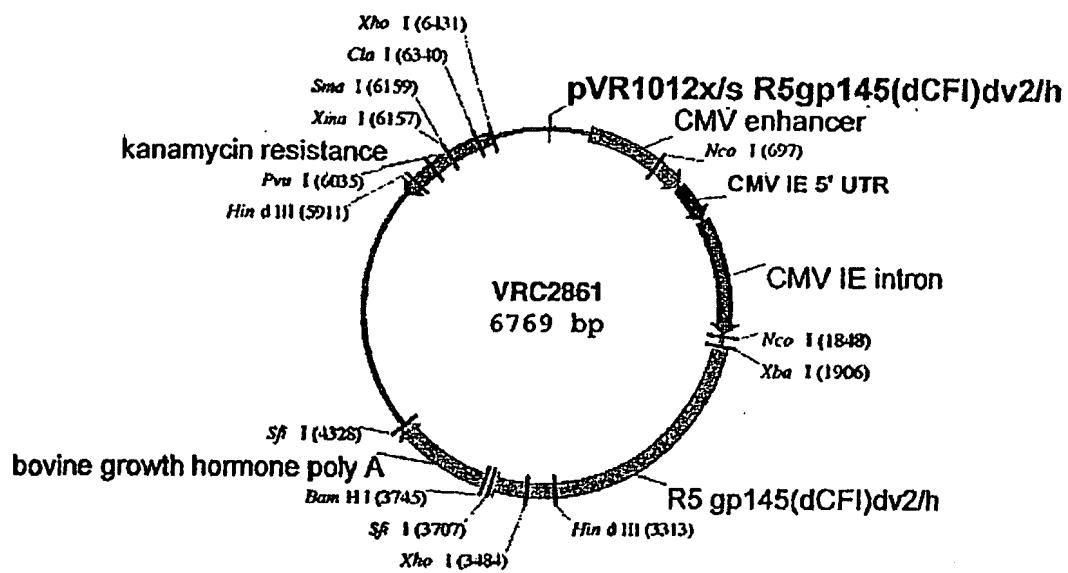


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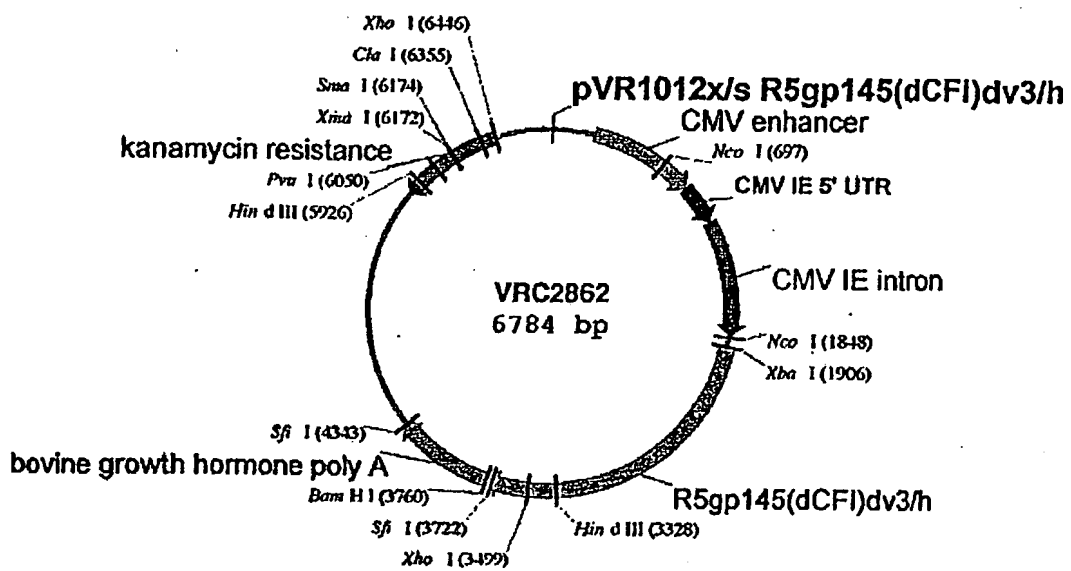


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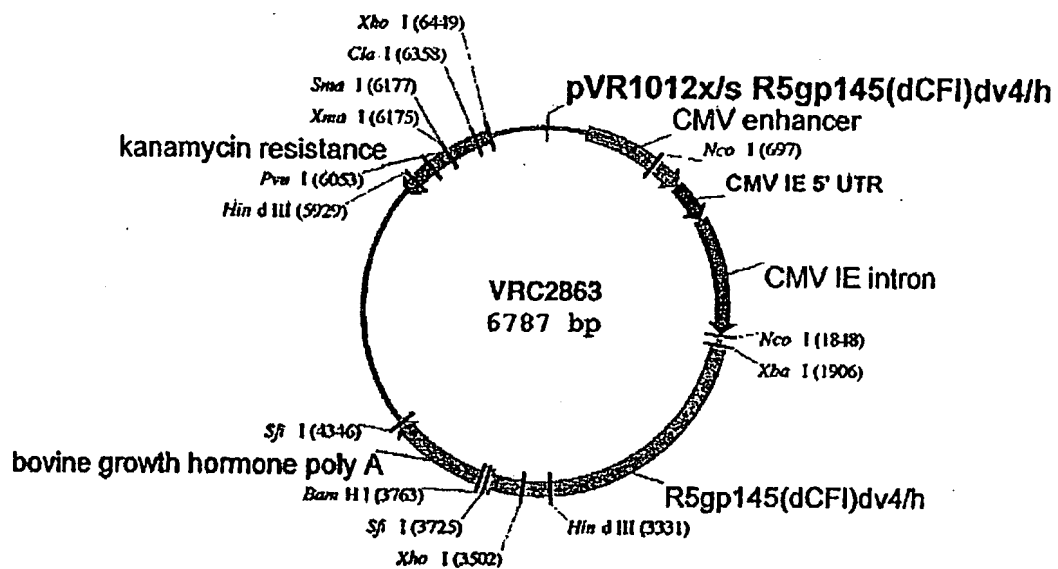


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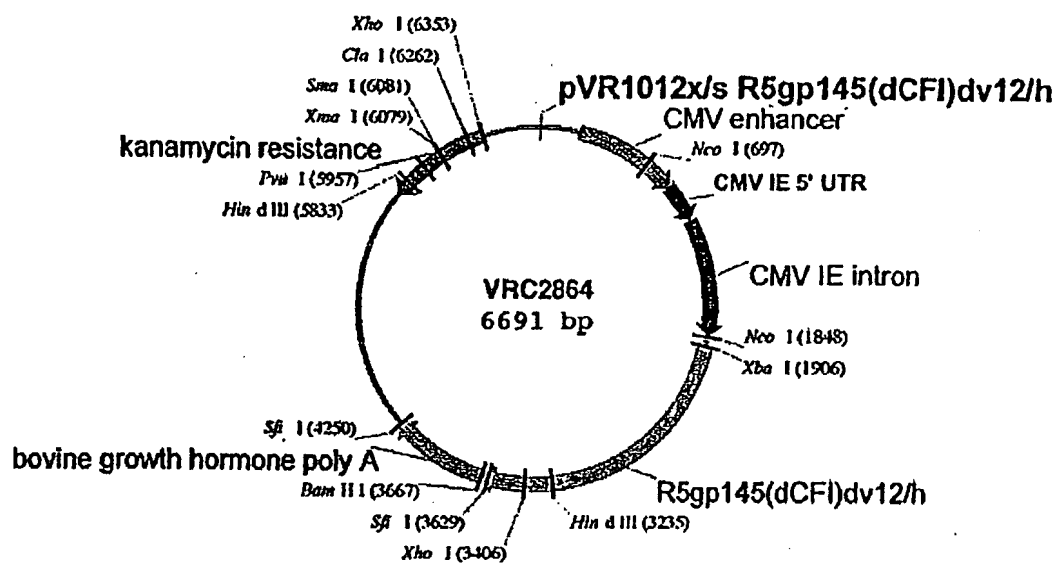


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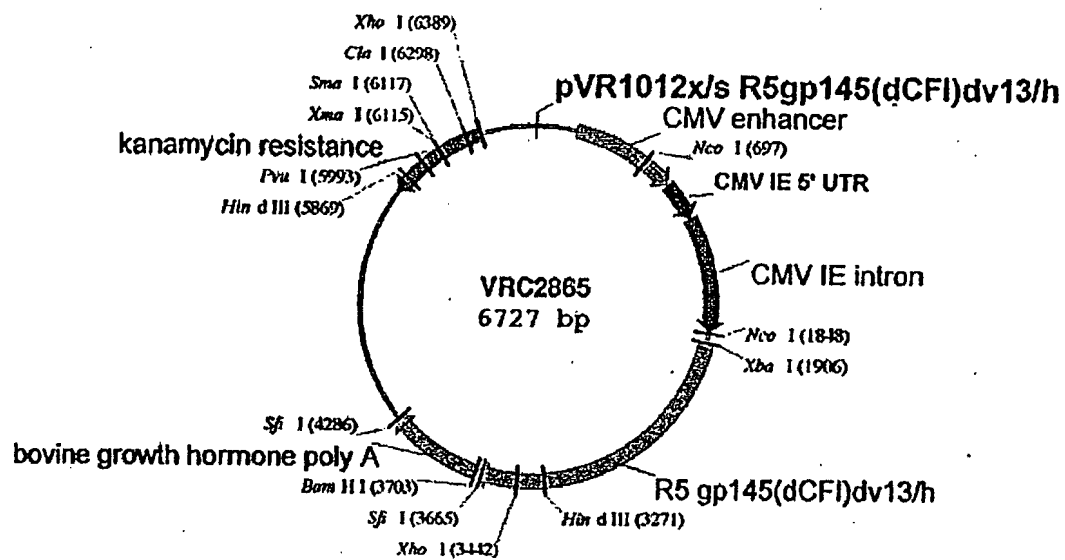


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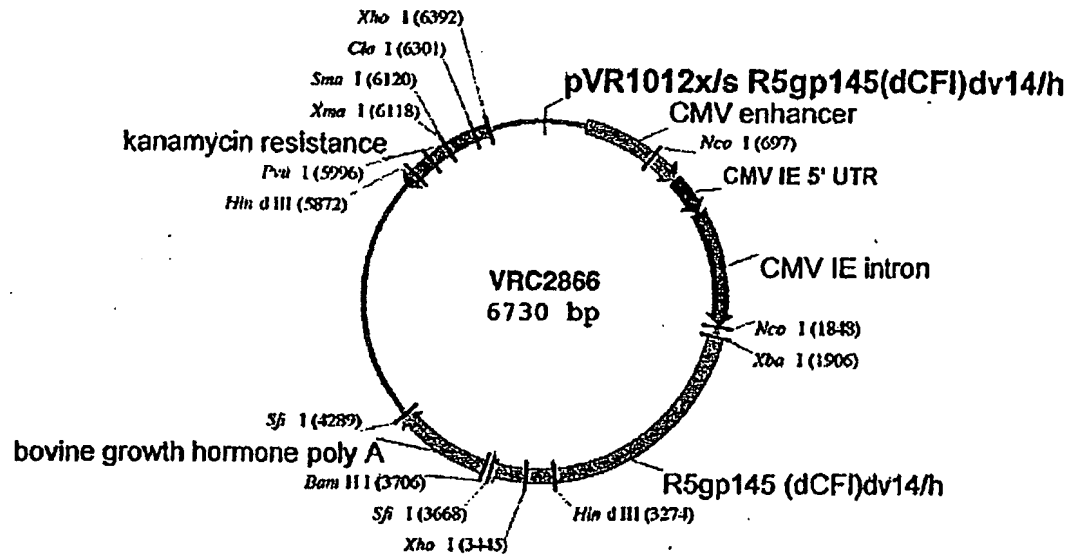


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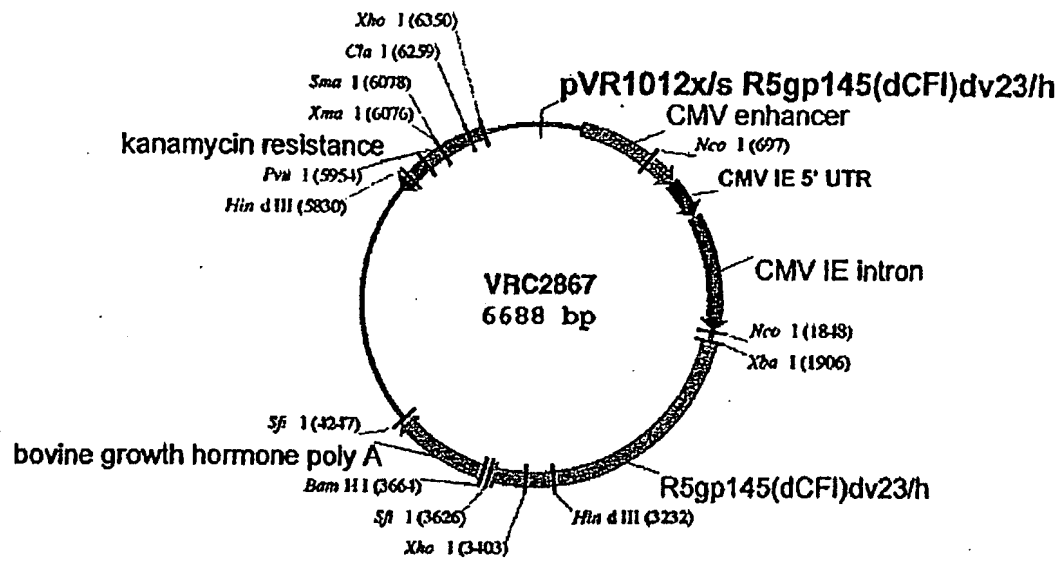


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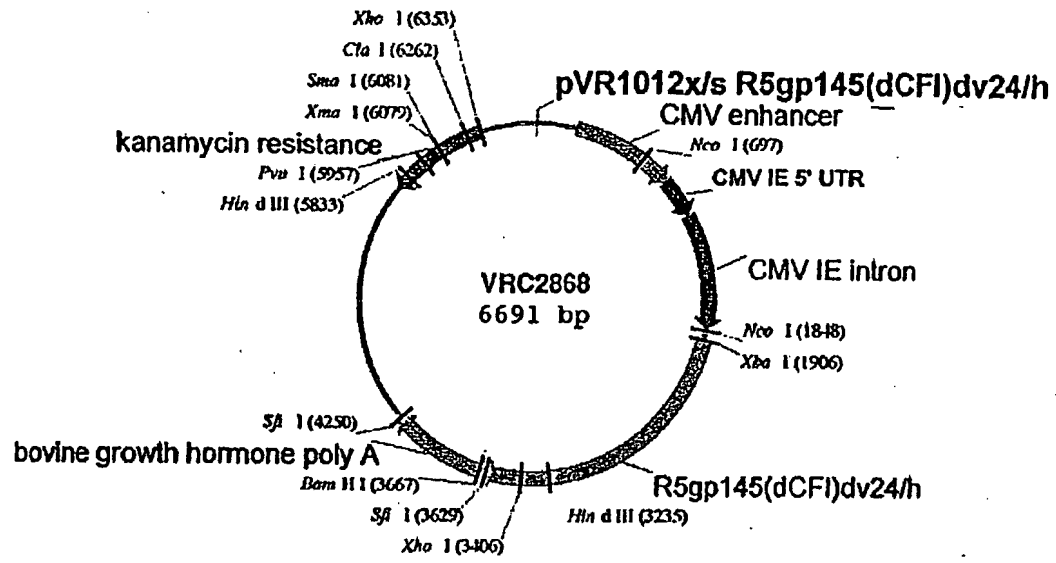


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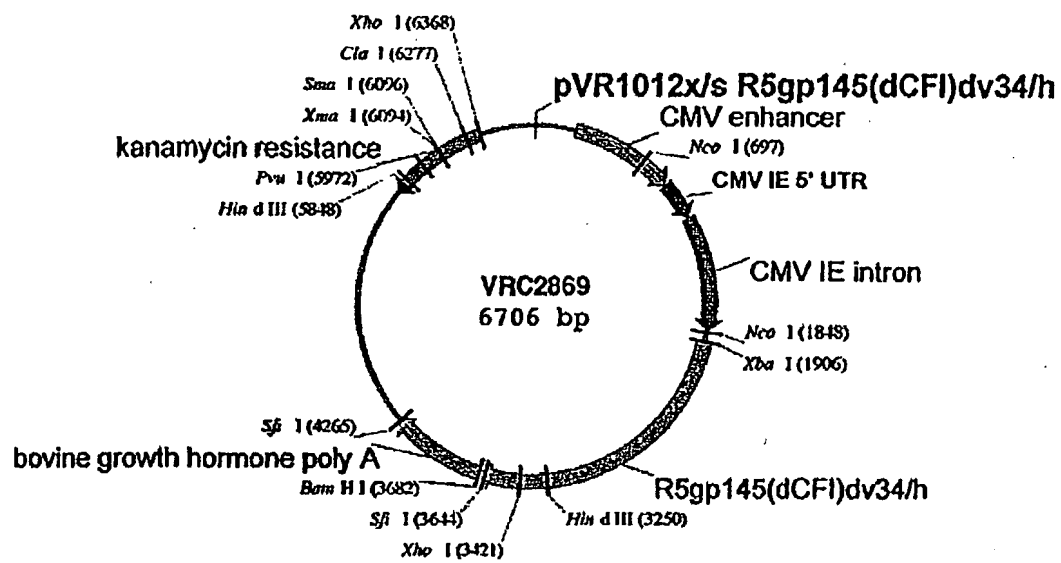


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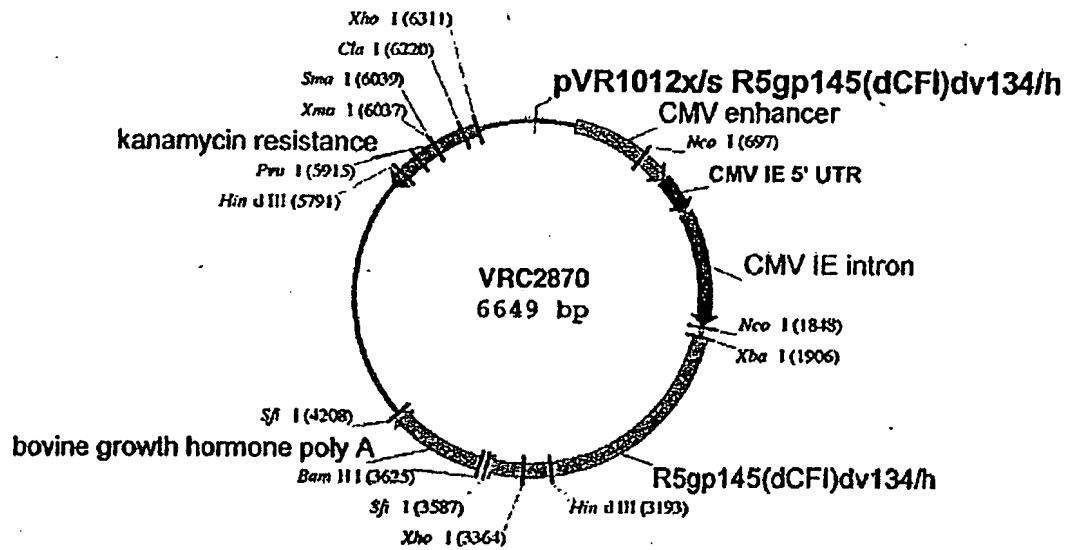


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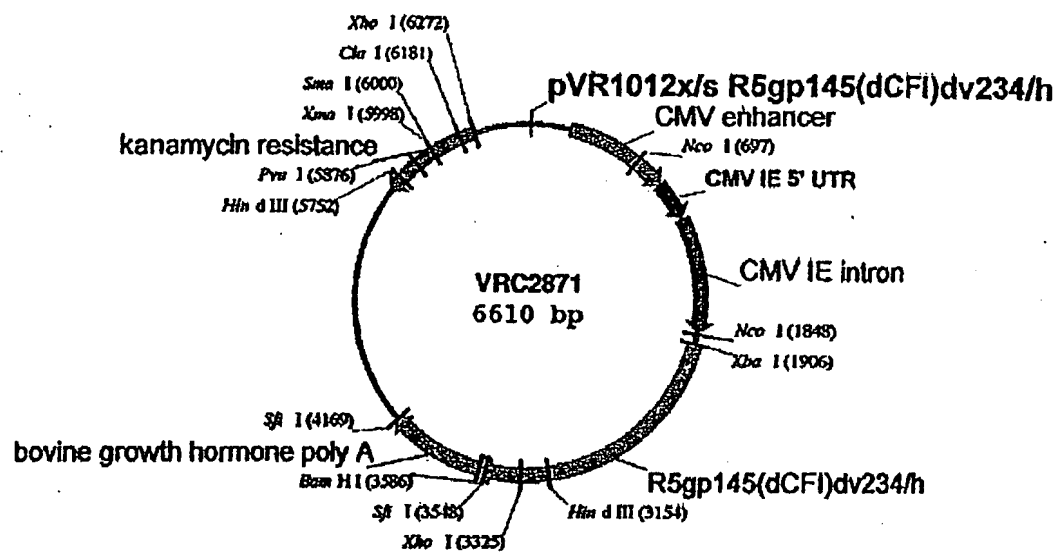


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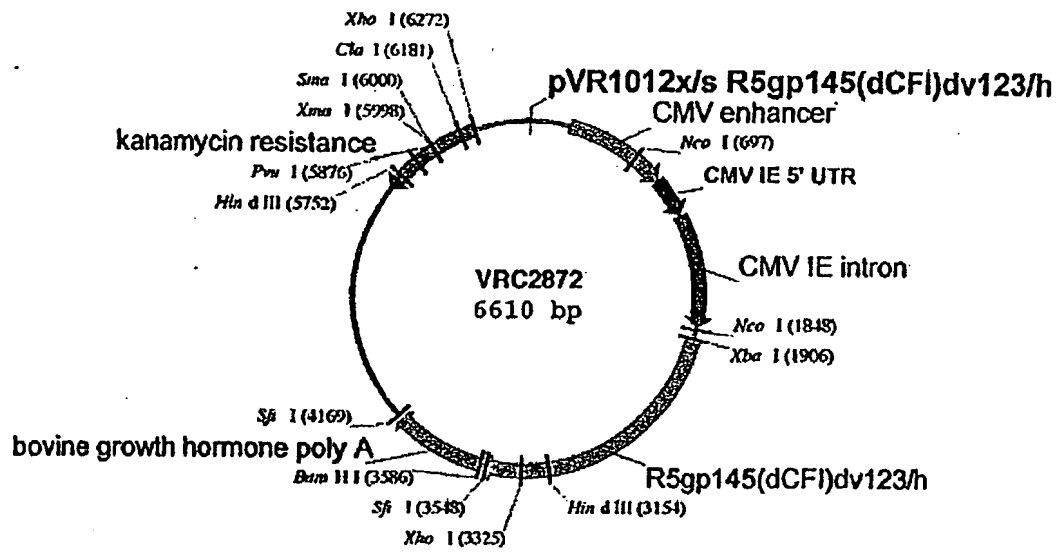


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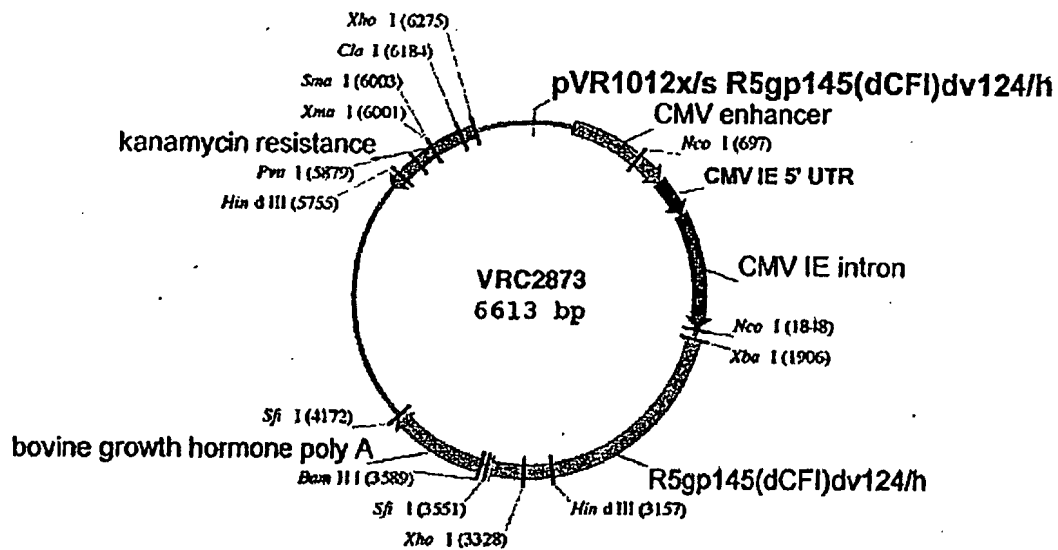


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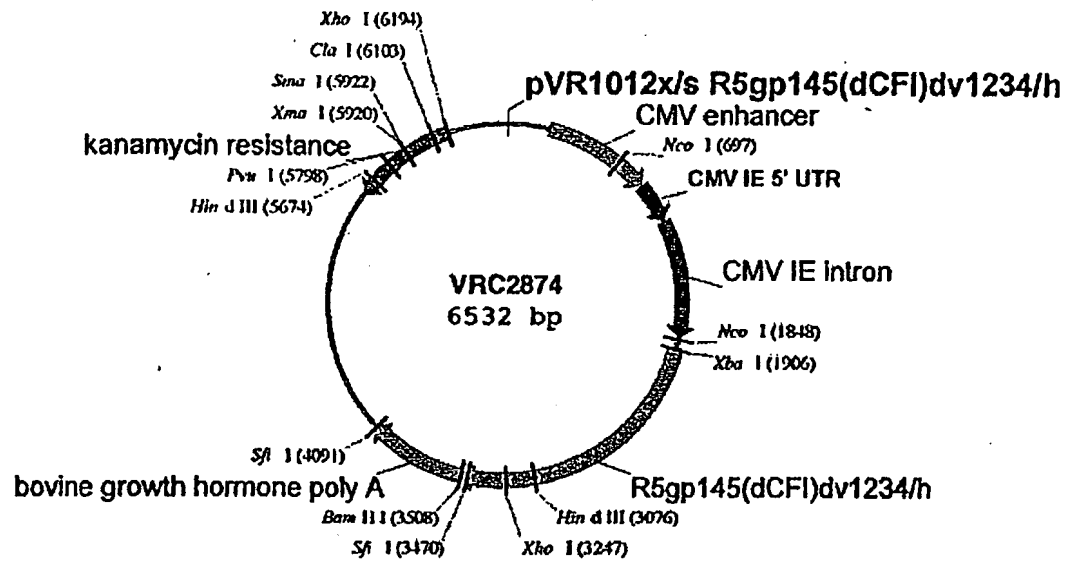


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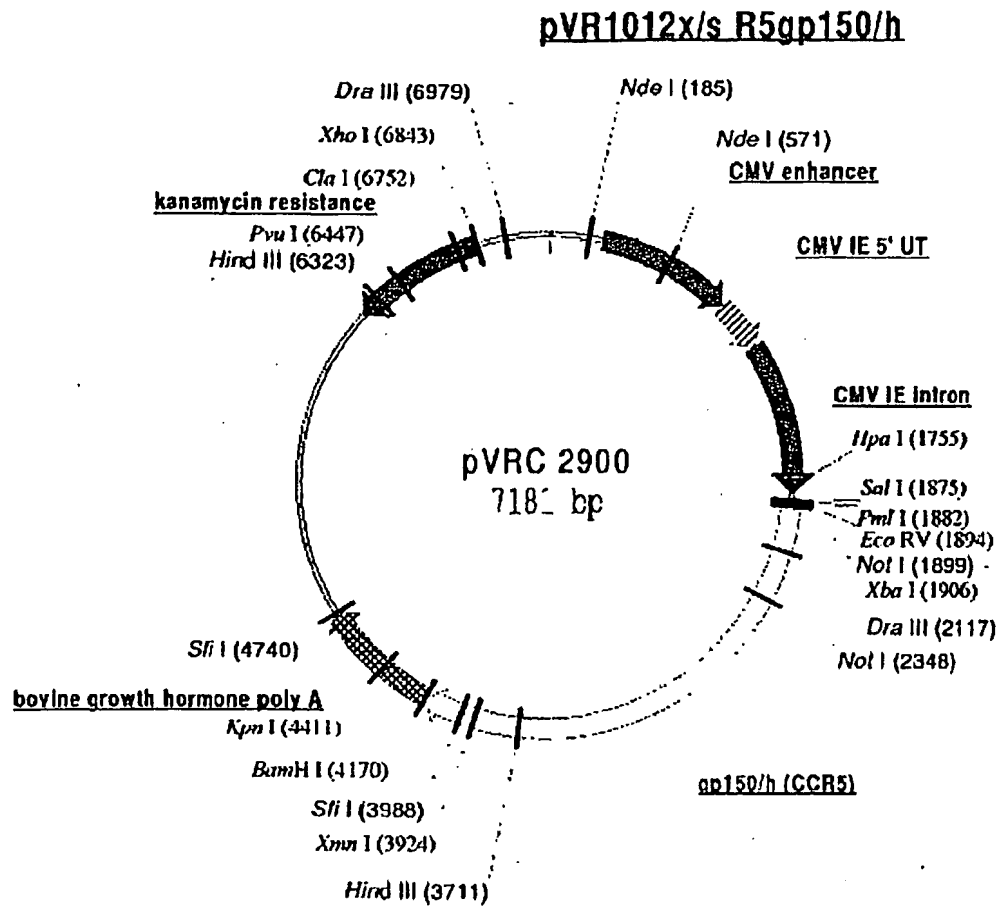


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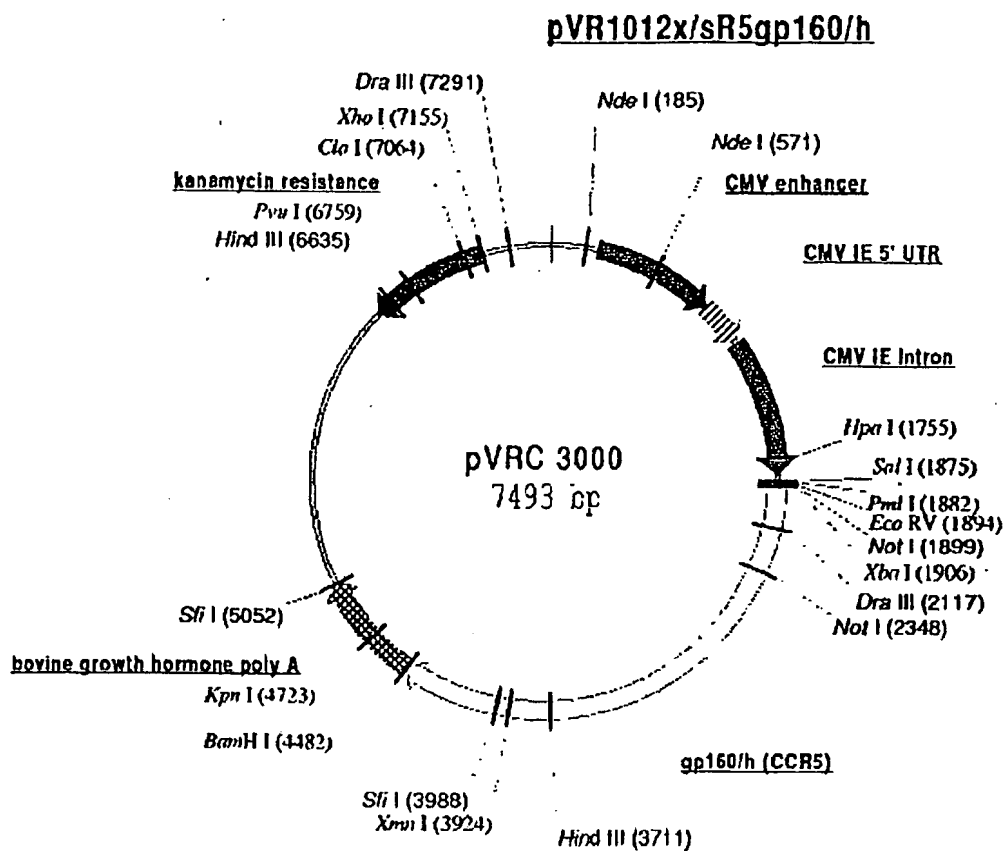


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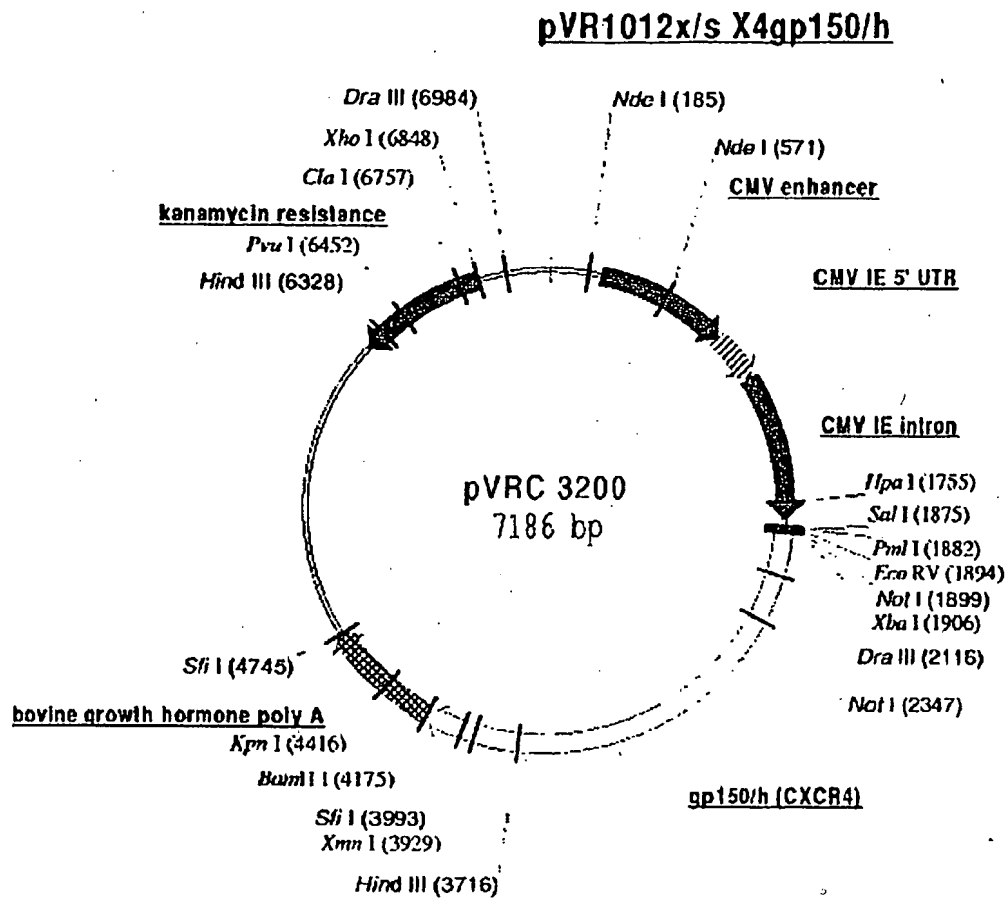


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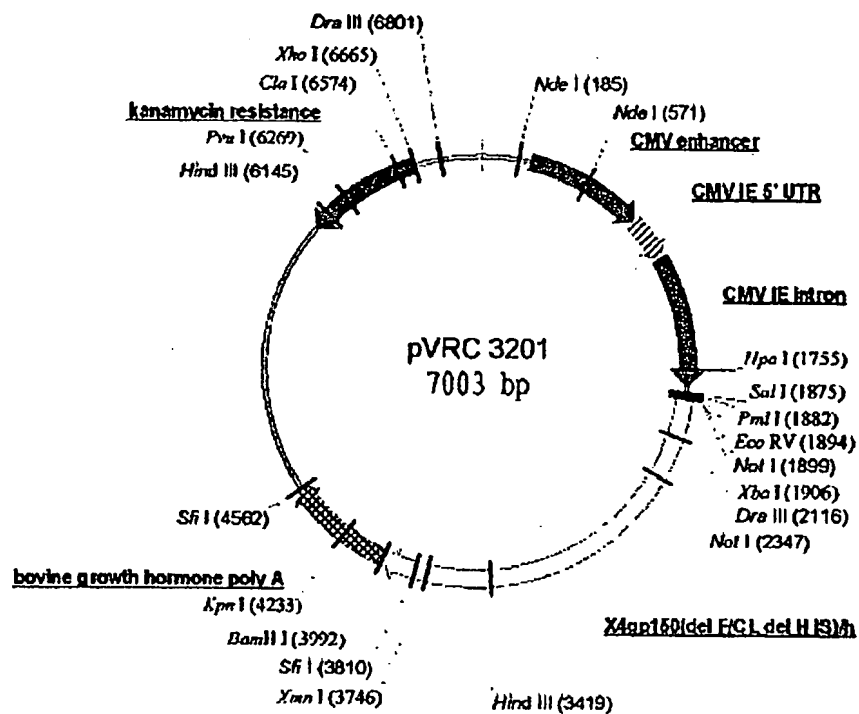
pVR1012x/s X4gp150(del F/CL del H IS)/h

Figure 73

pVR1012x/s.X4gp150_delta.gly

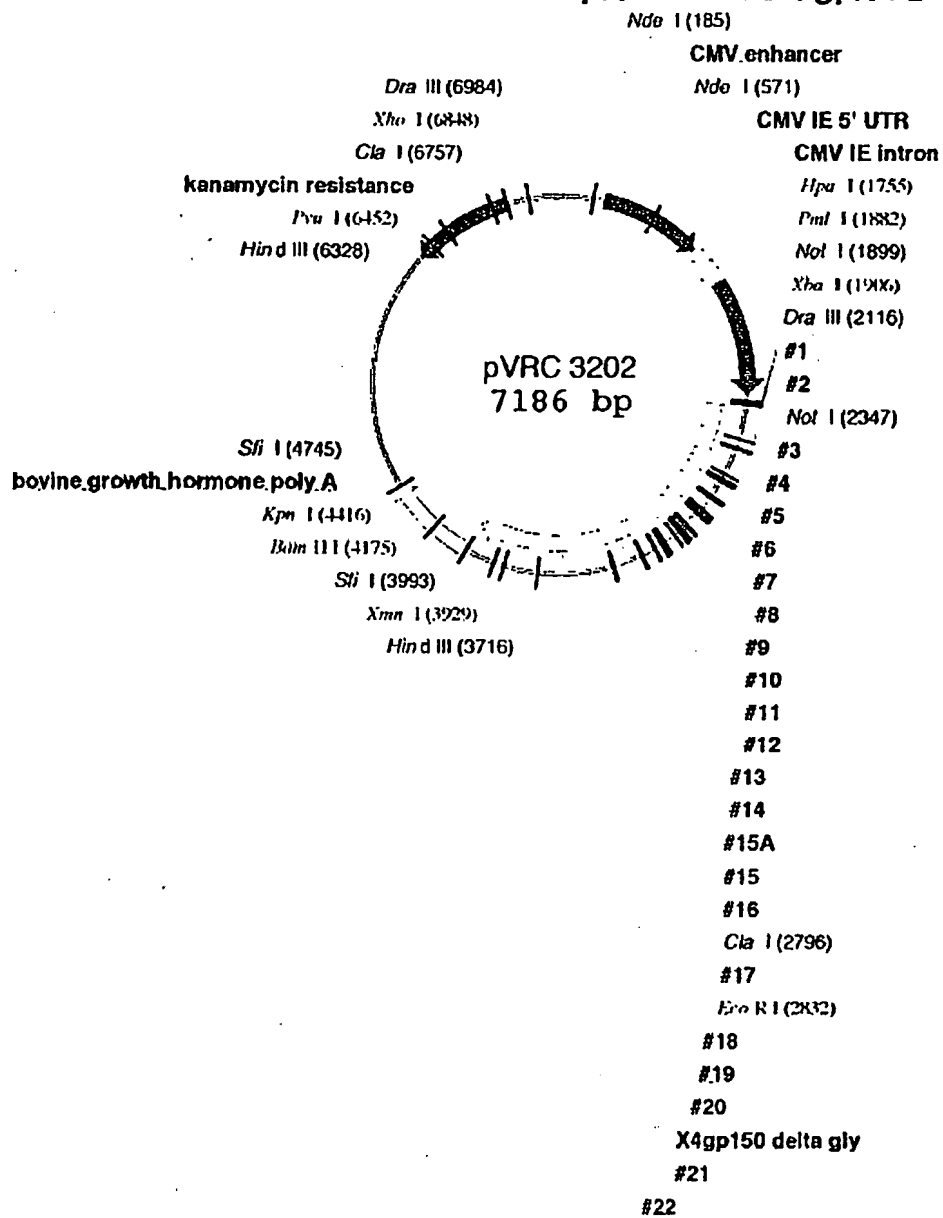


Figure 74

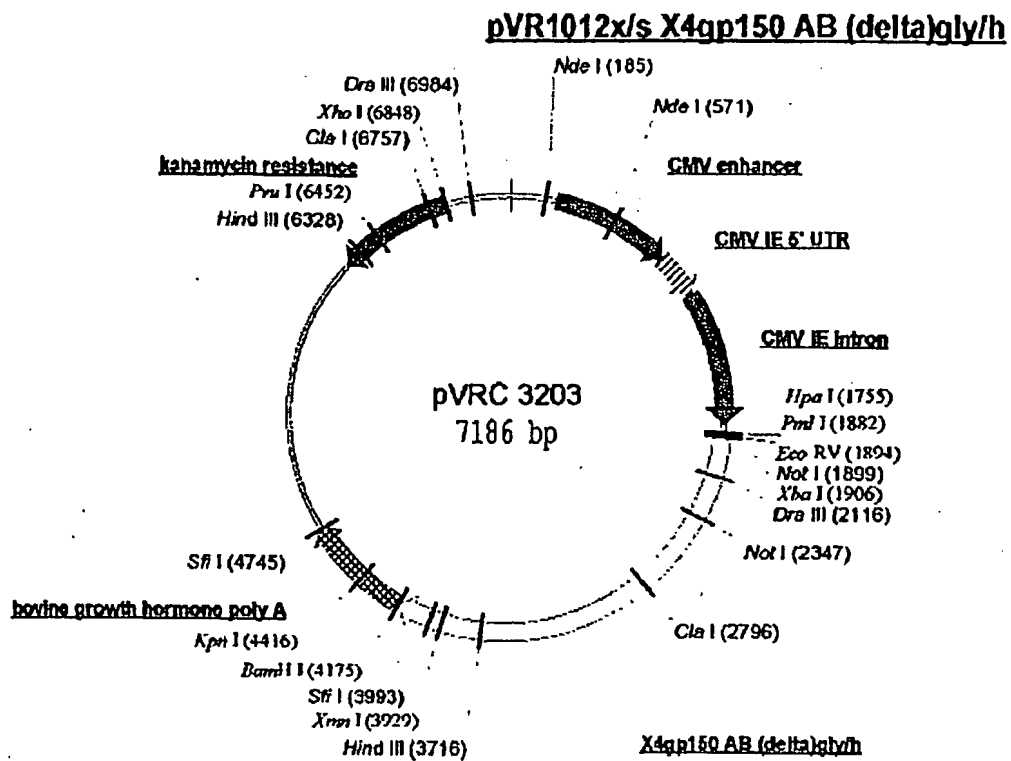


Figure 75

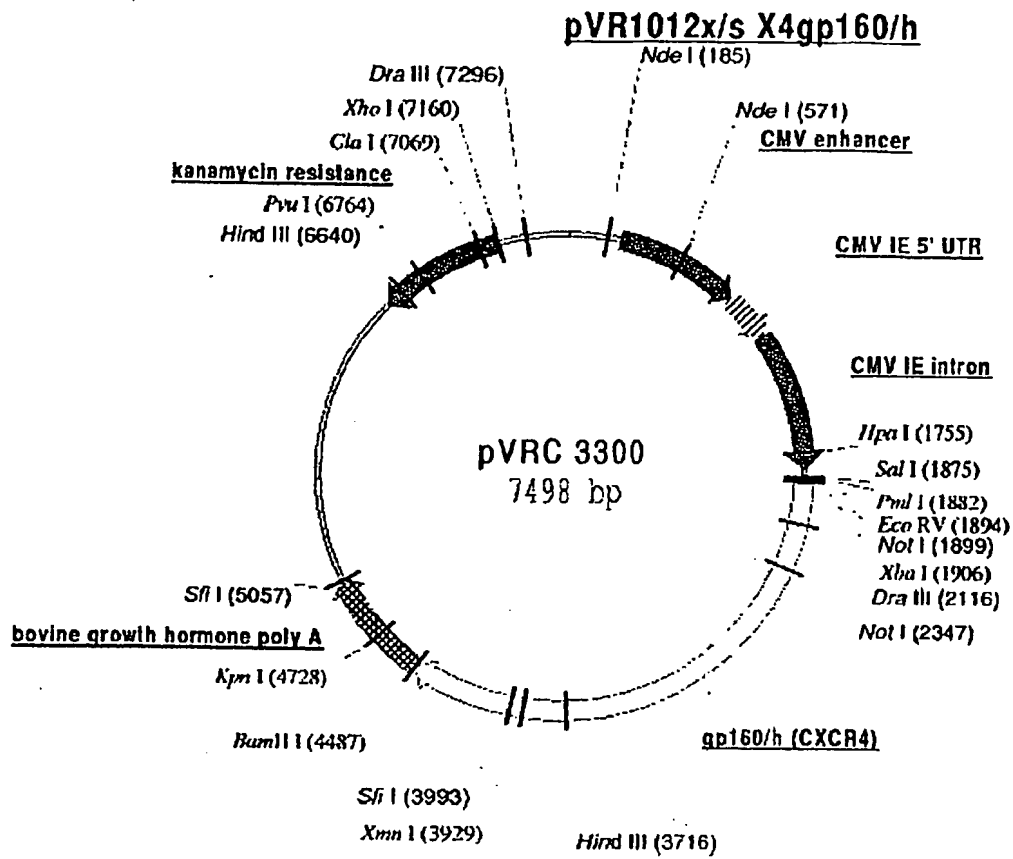


Figure 76

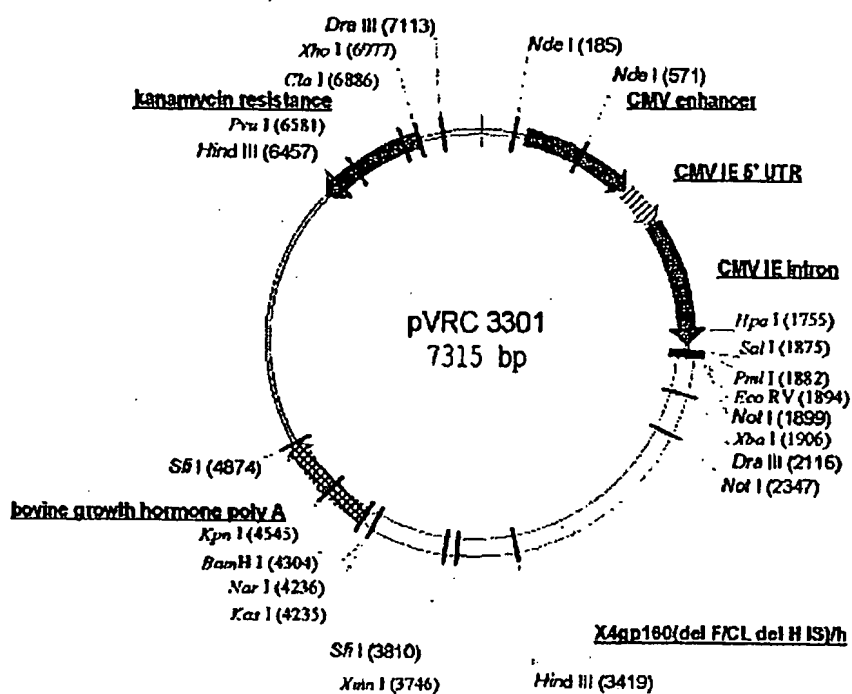
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Figure 77

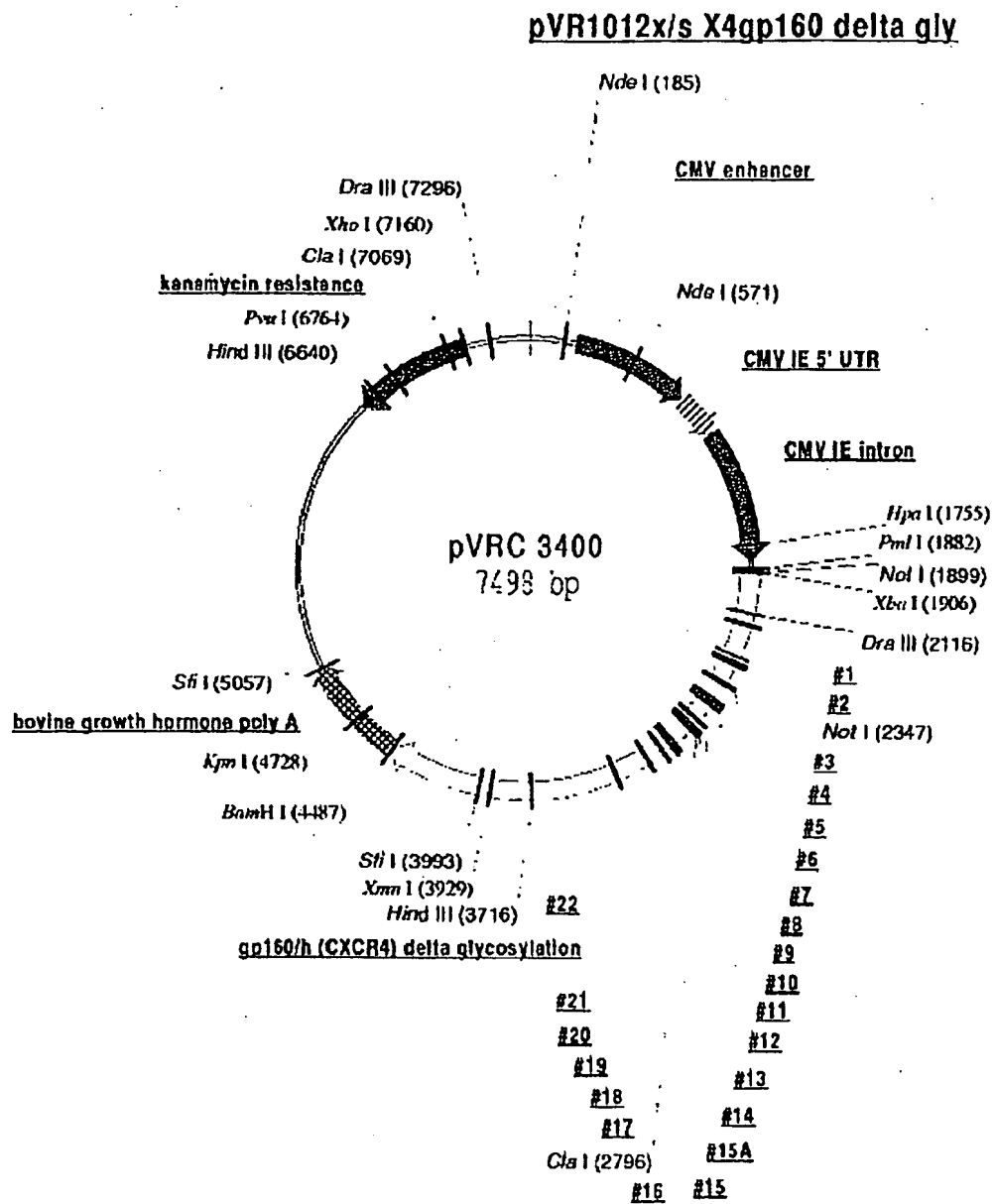


Figure 78

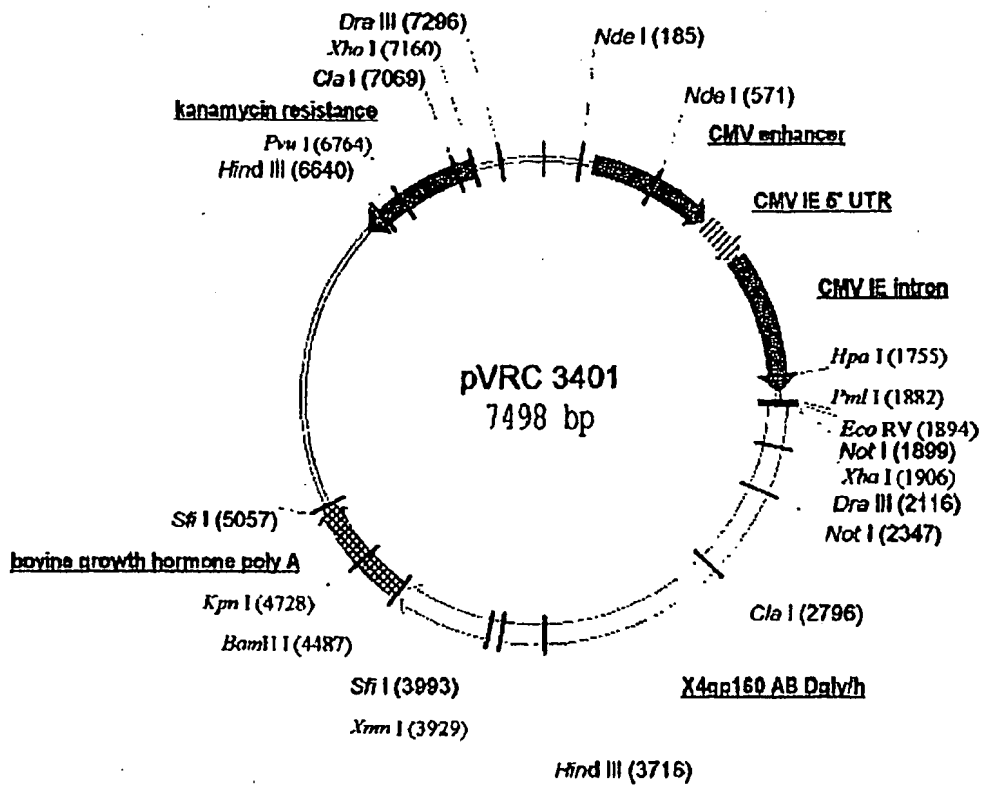
pVR1012x/s X4gp160 AB Dqly/h

Figure 79

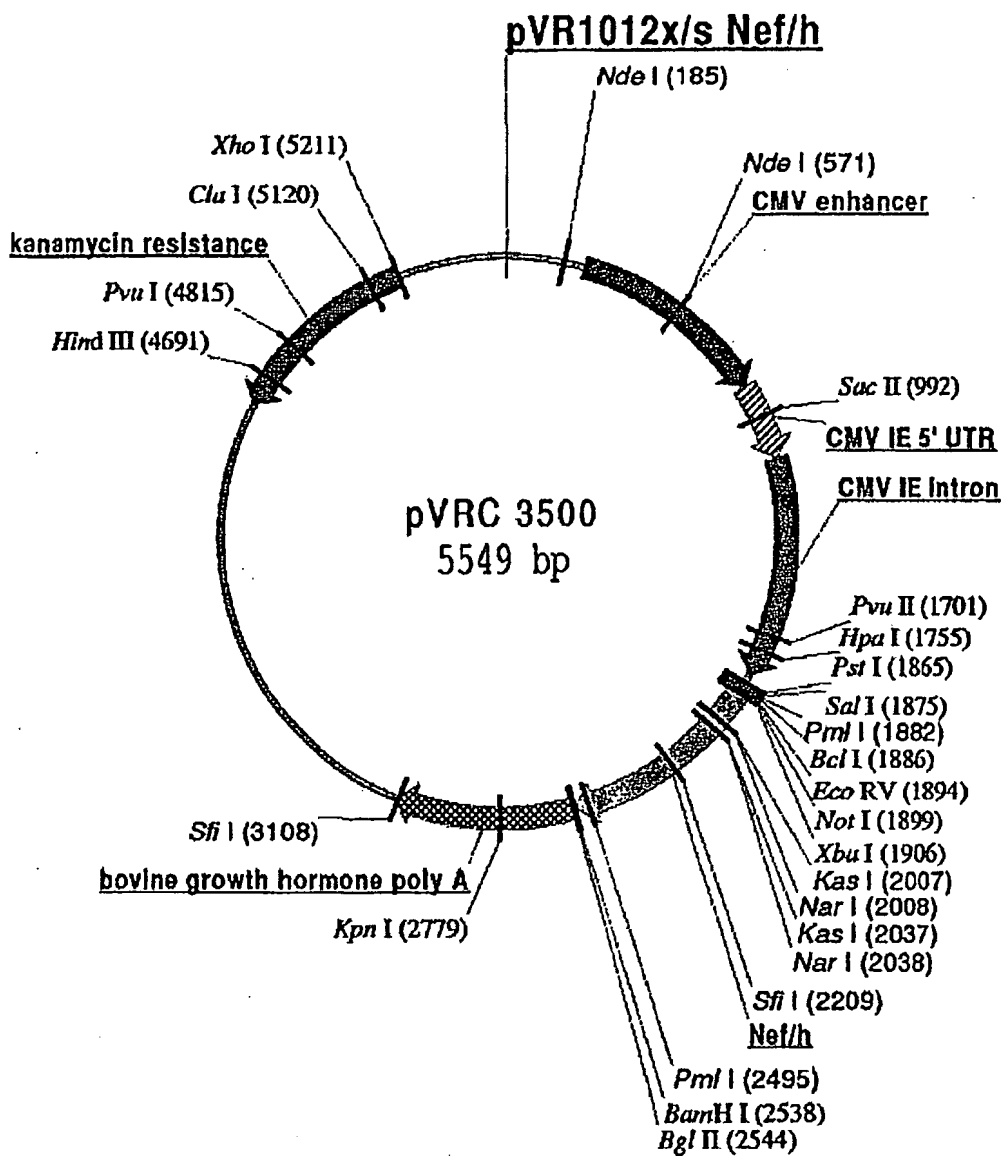


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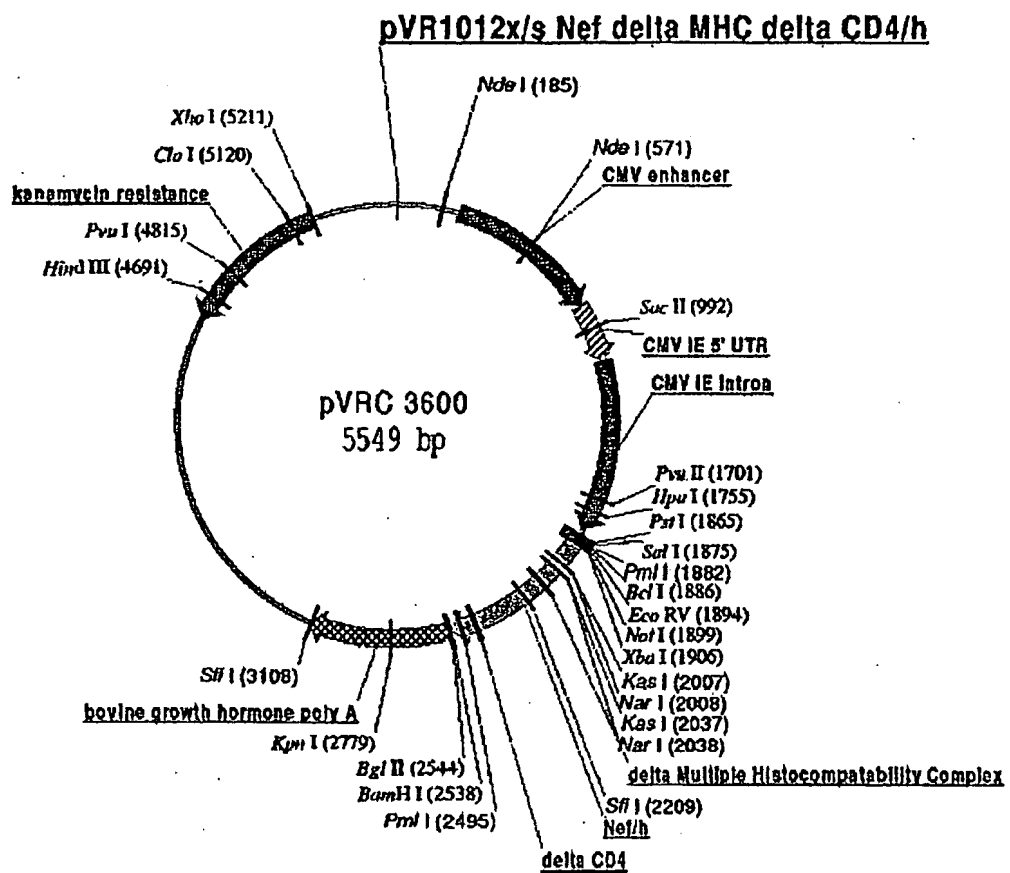


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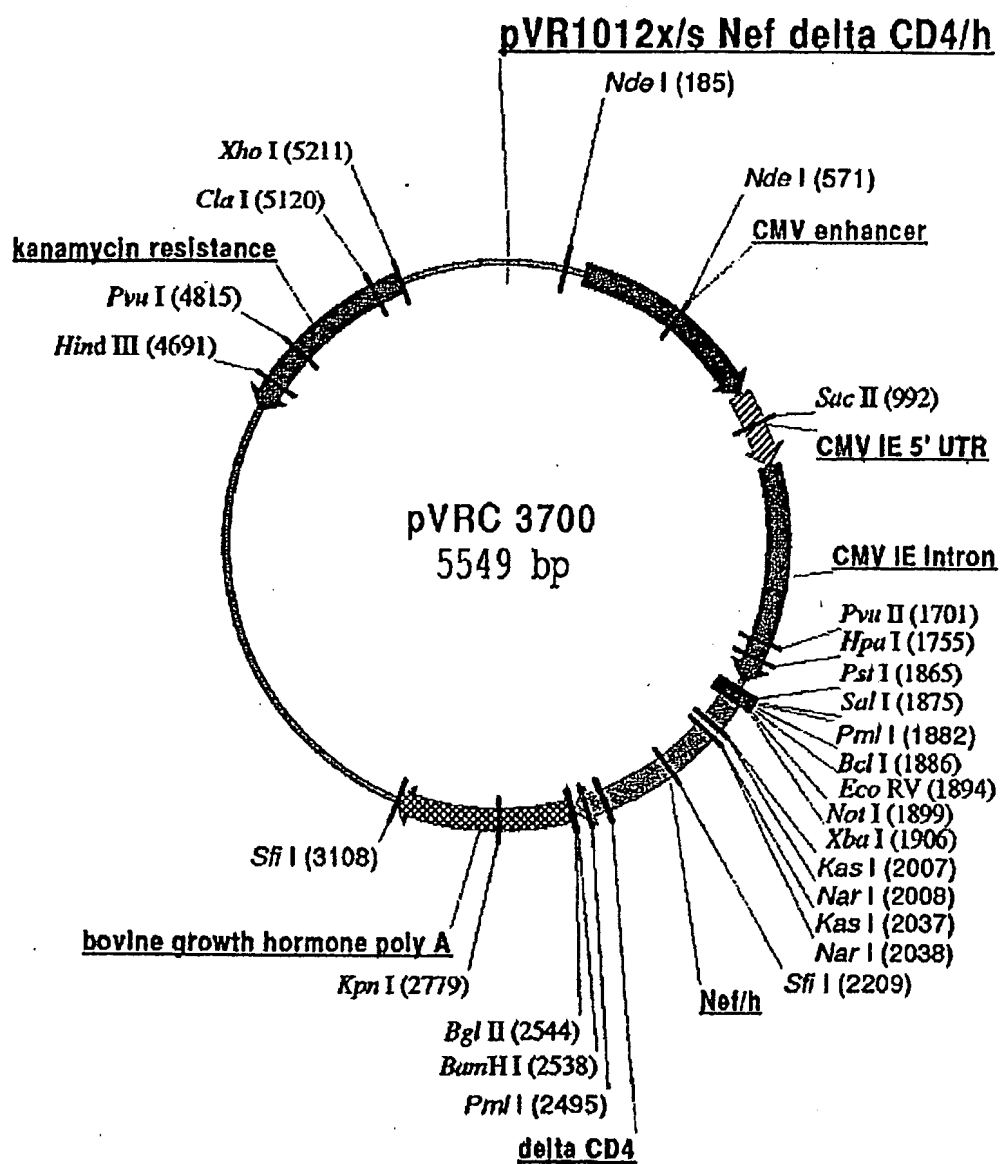


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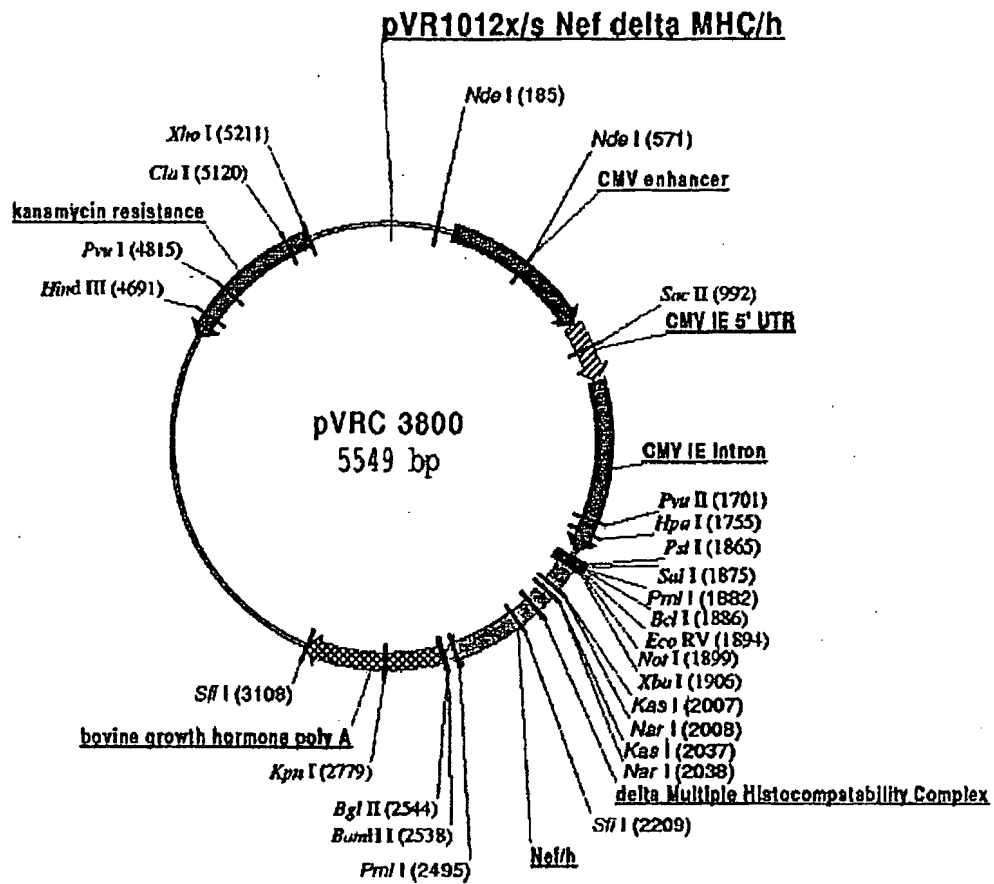


Figure 83

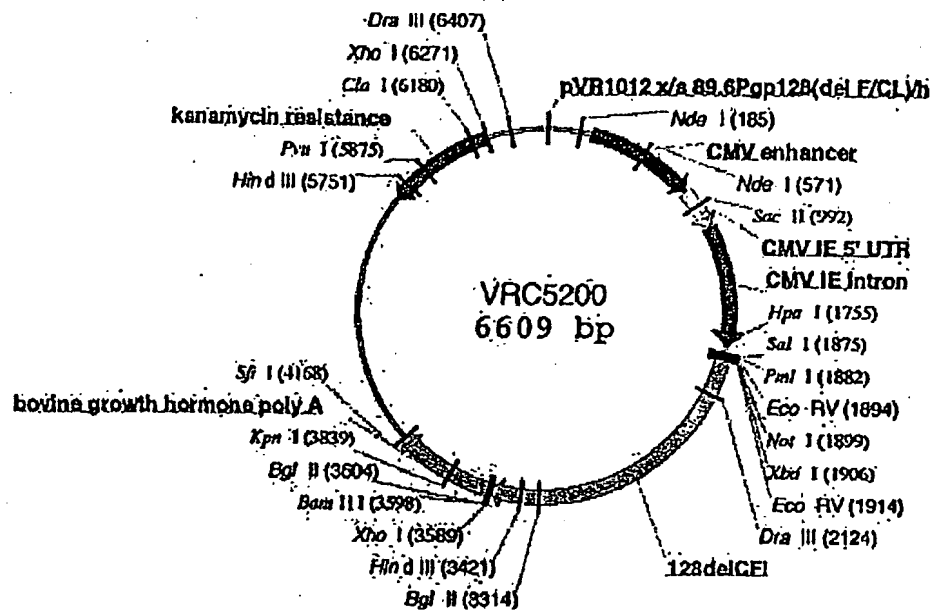


Figure 84

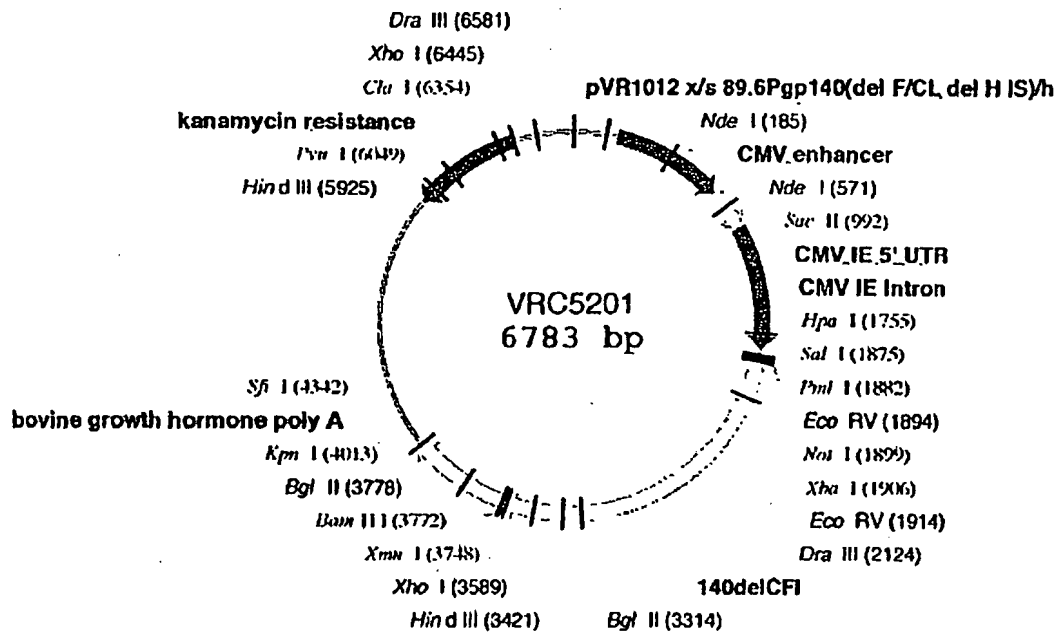


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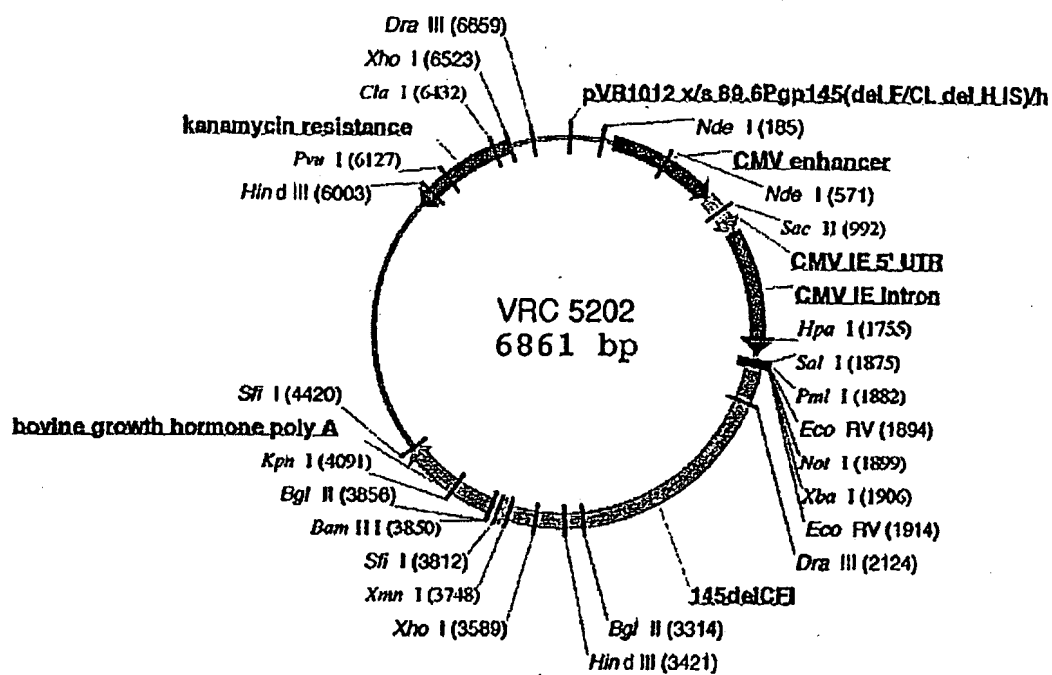


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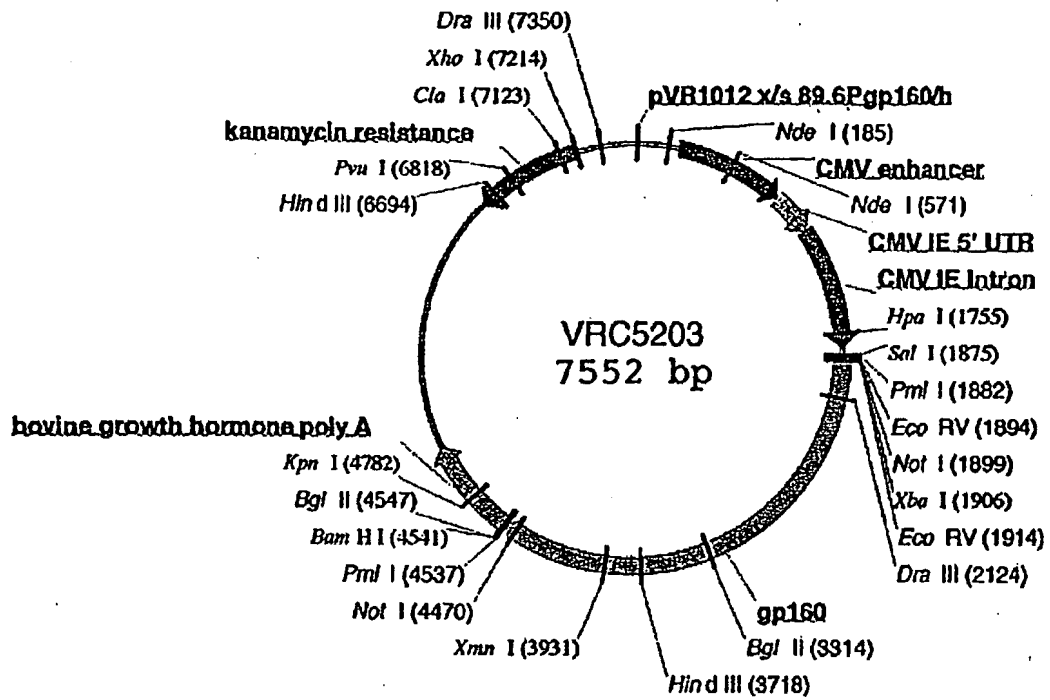


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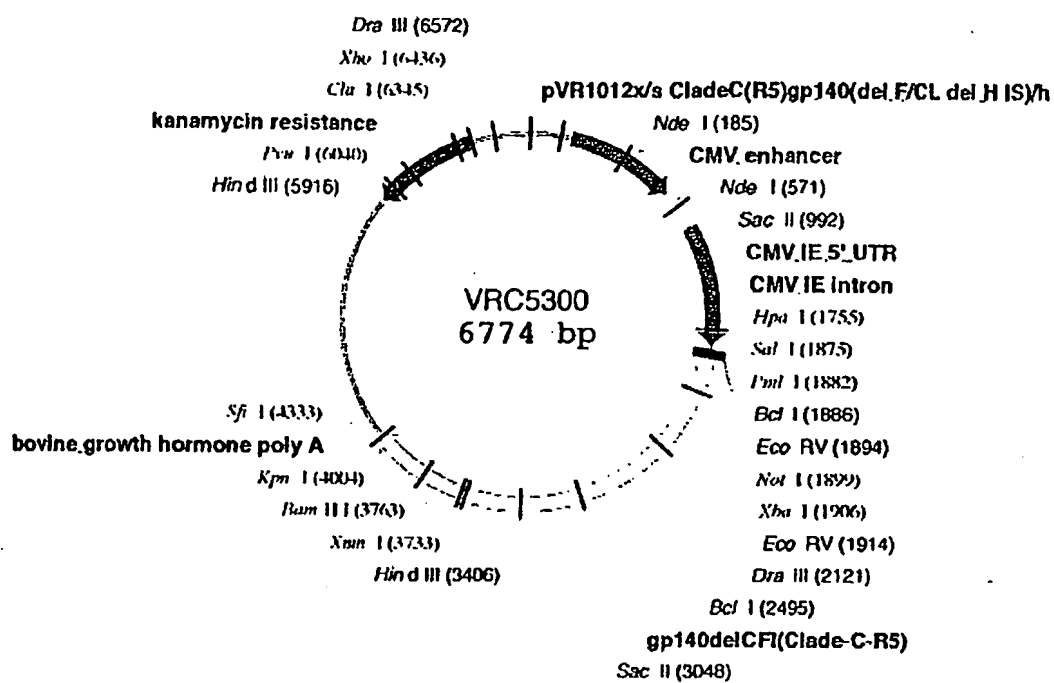


Figure 88

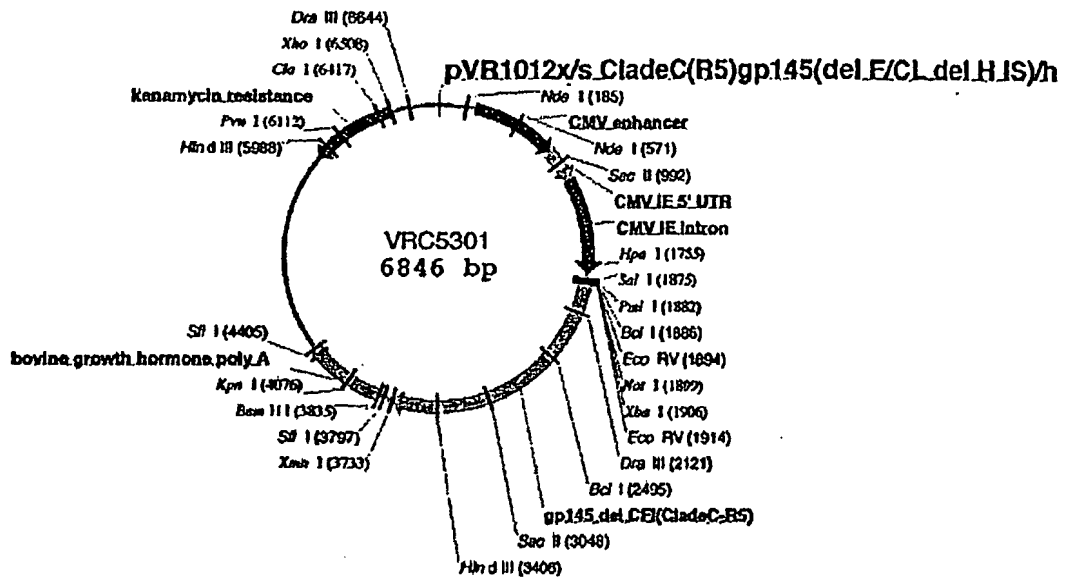


Figure 89

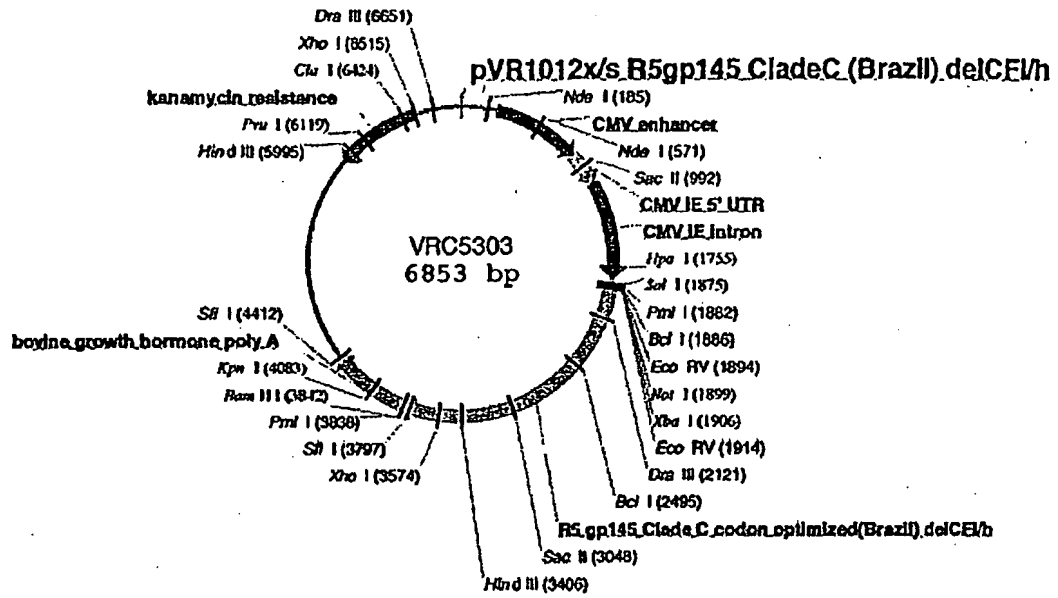


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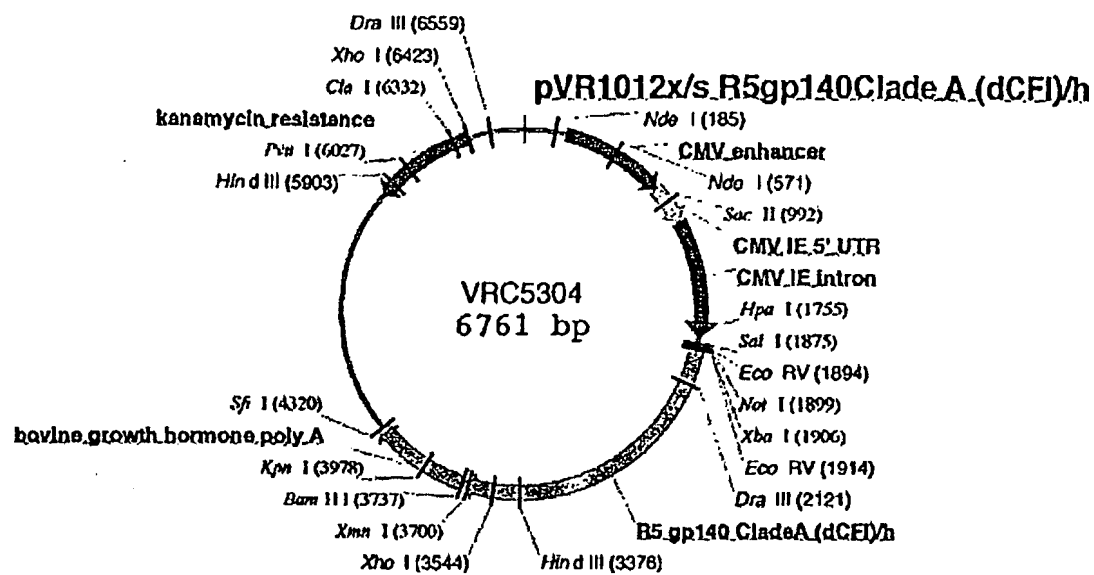


Figure 91

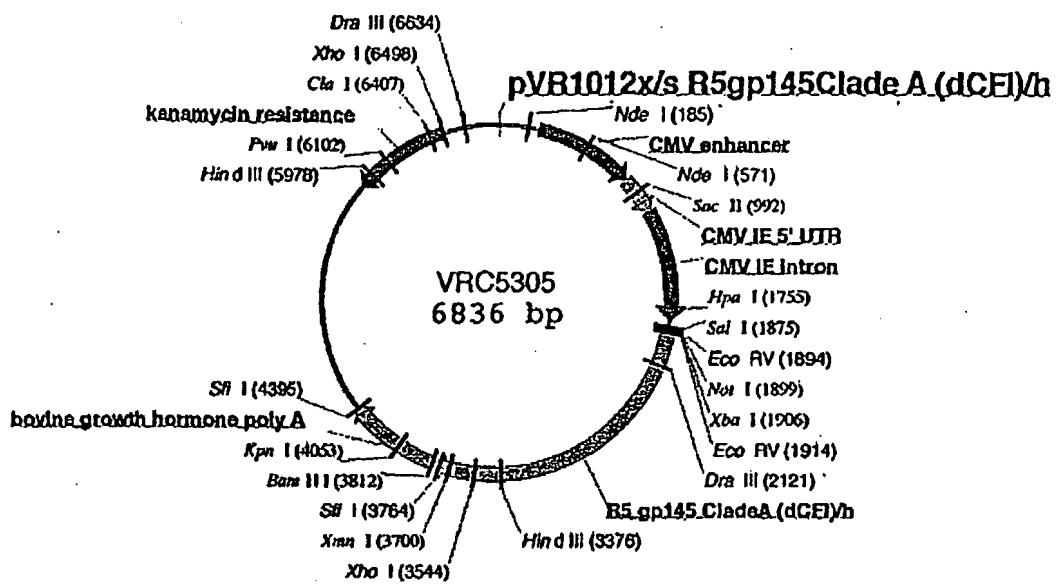


Figure 92

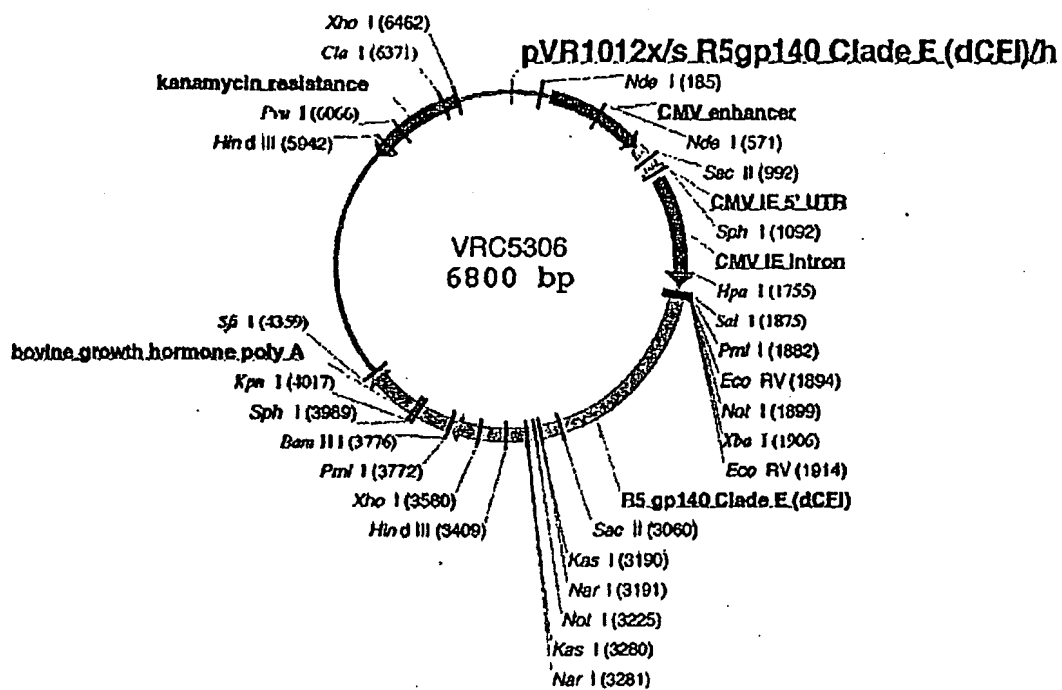


Figure 93

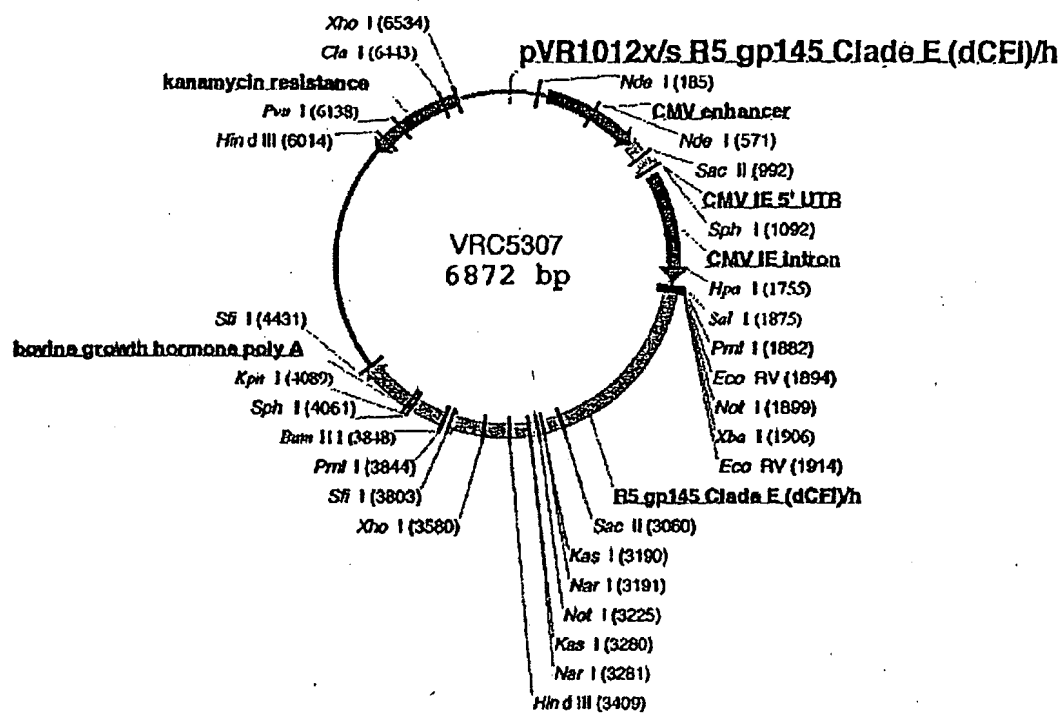


Figure 94

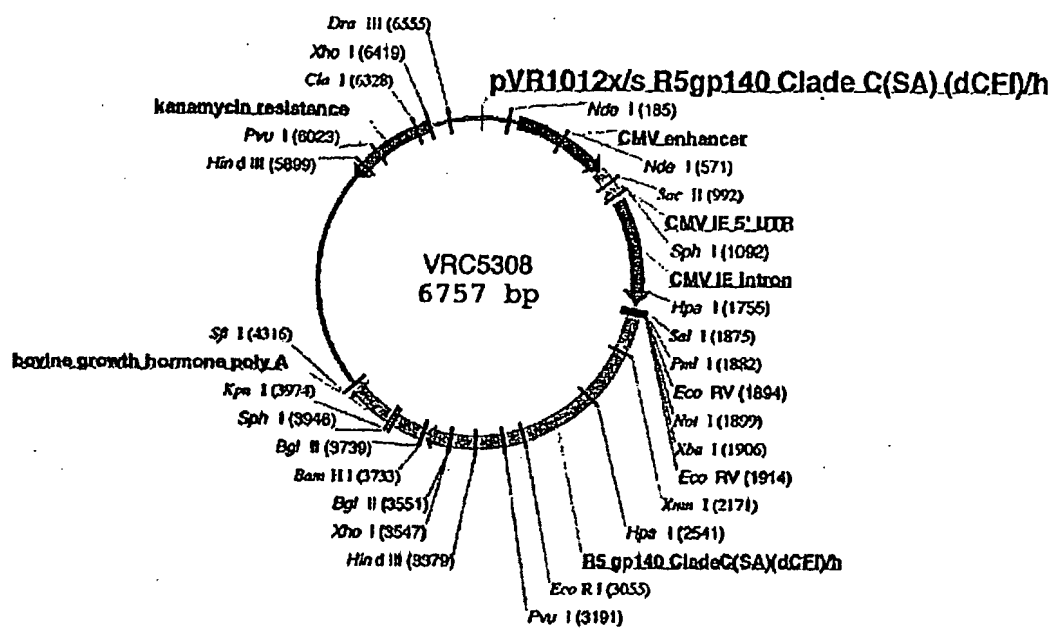


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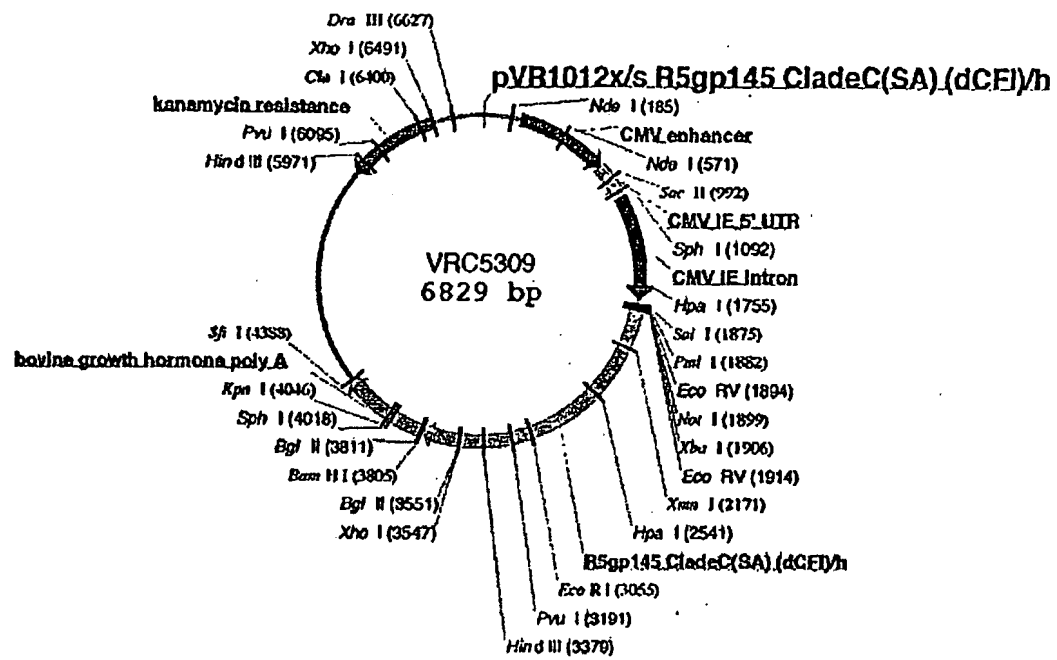


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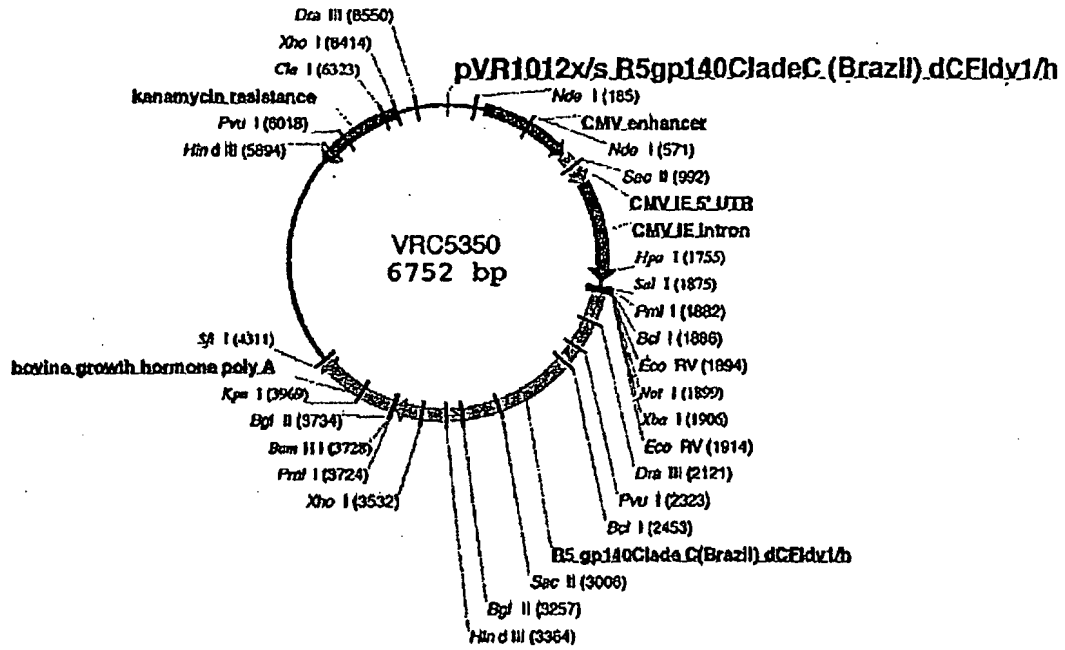


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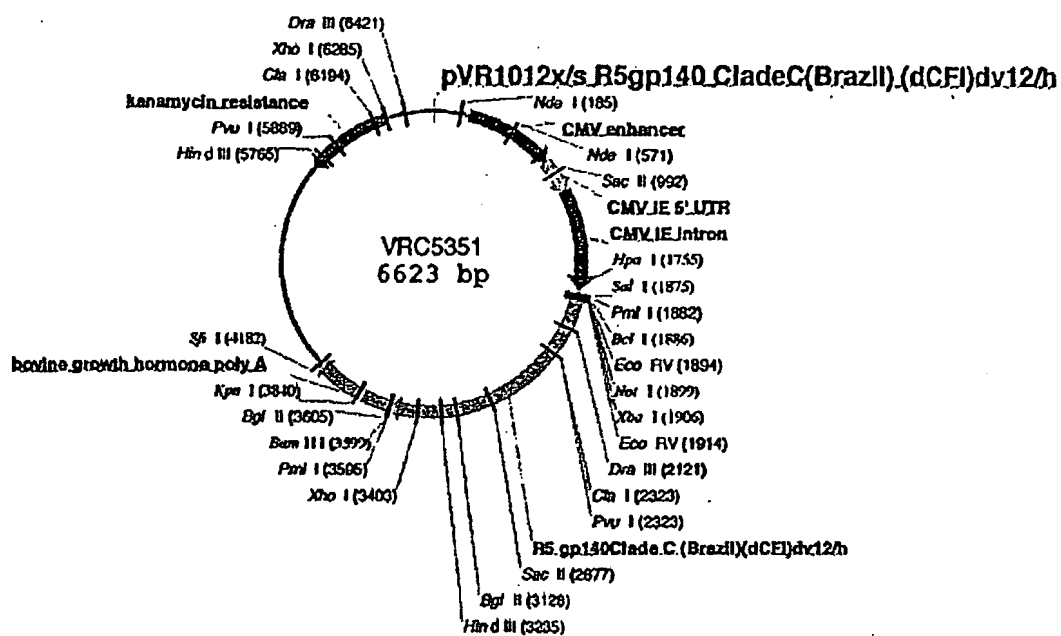


Figure 98

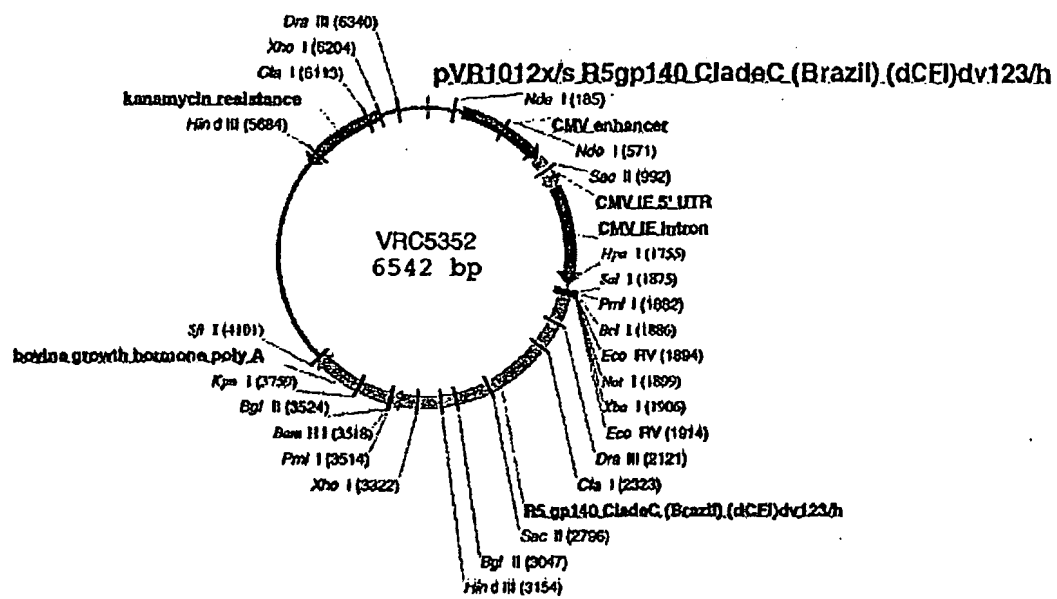


Figure 99

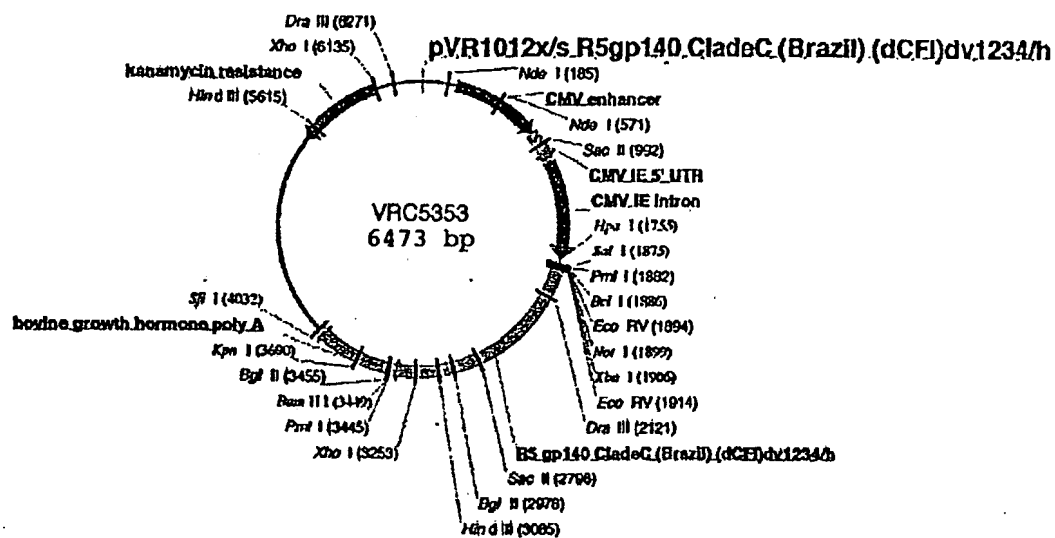


Figure 100

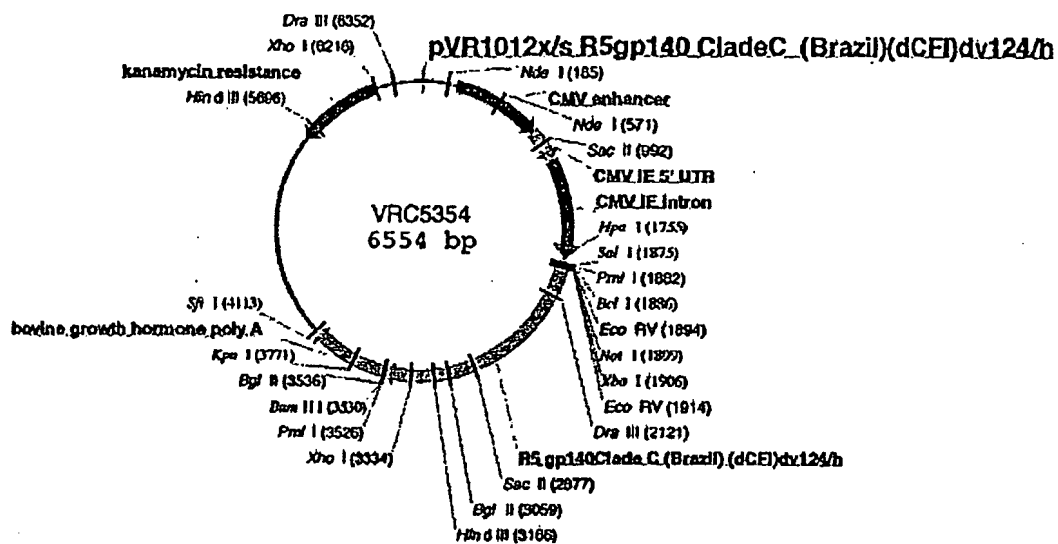


Figure 101

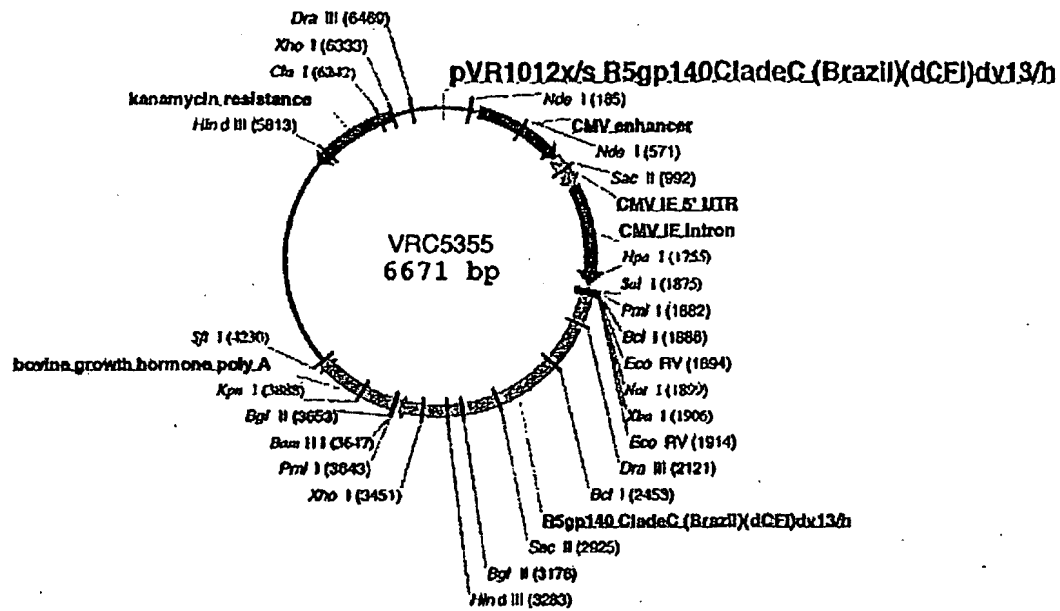


Figure 102

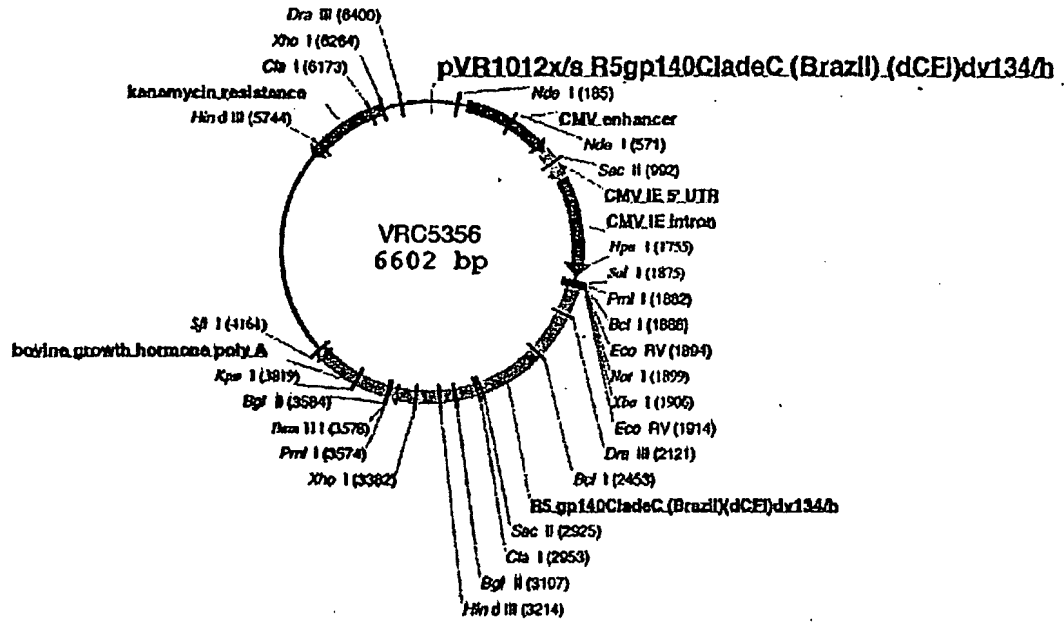


Figure 103

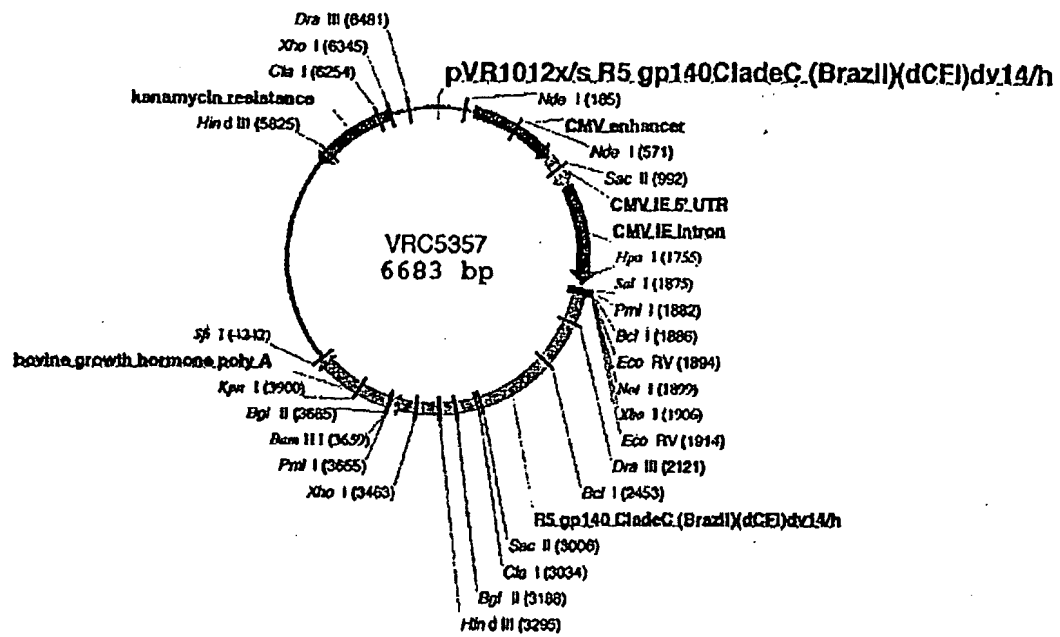


Figure 104

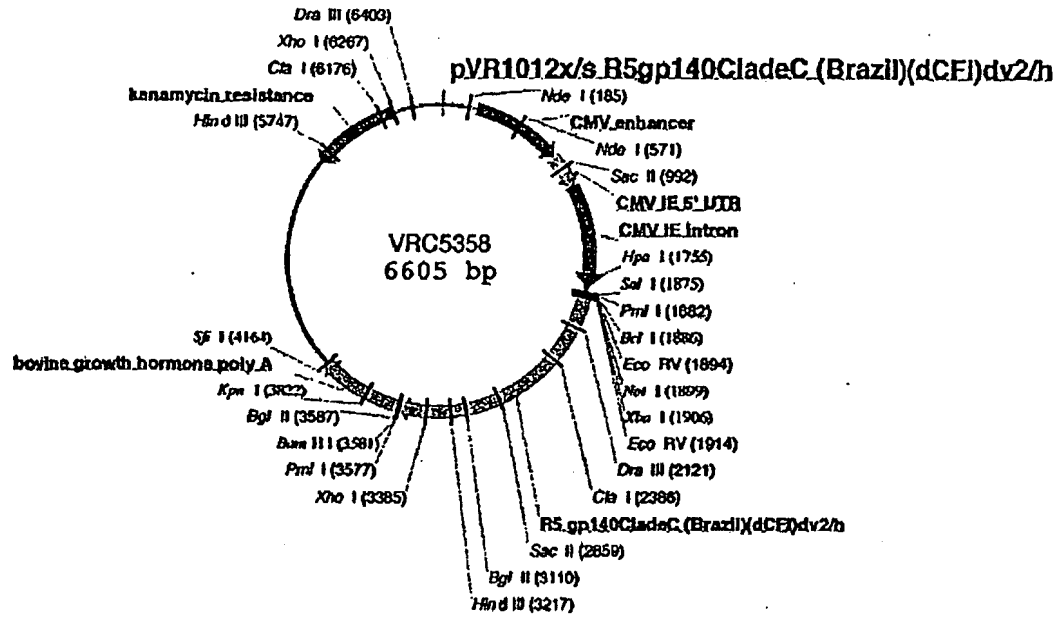


Figure 105

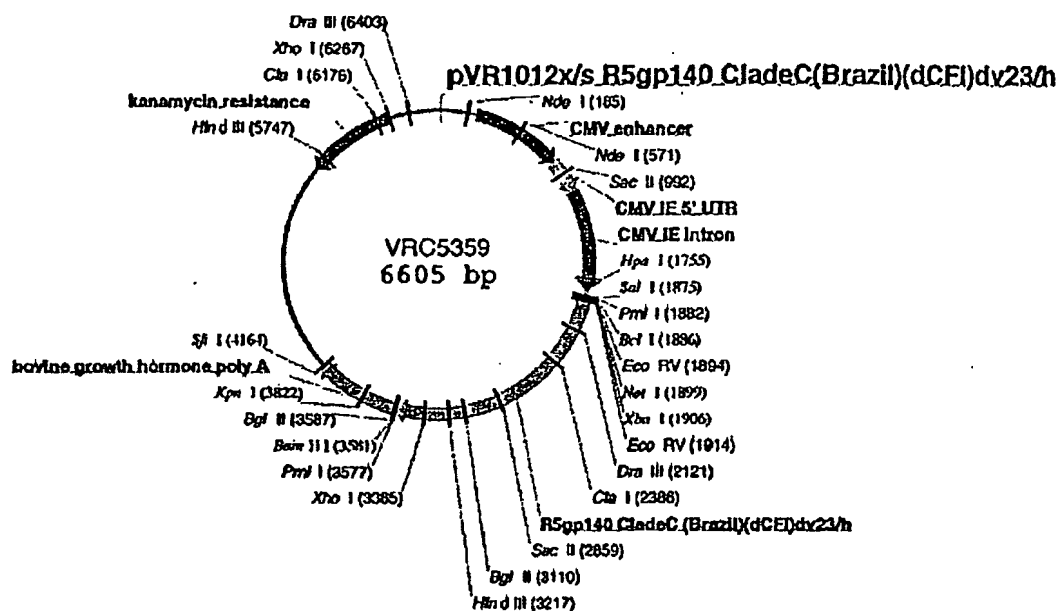


Figure 106

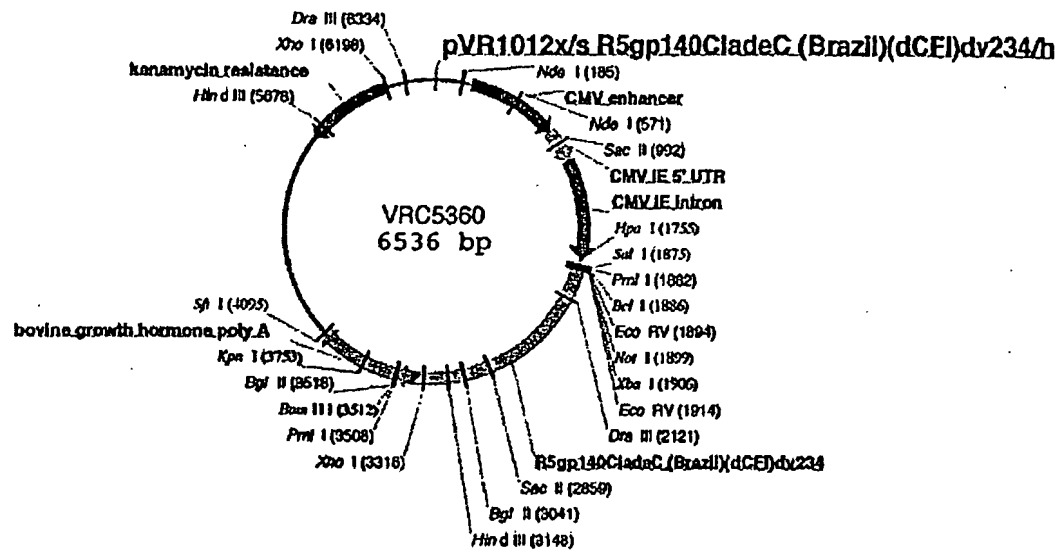


Figure 107

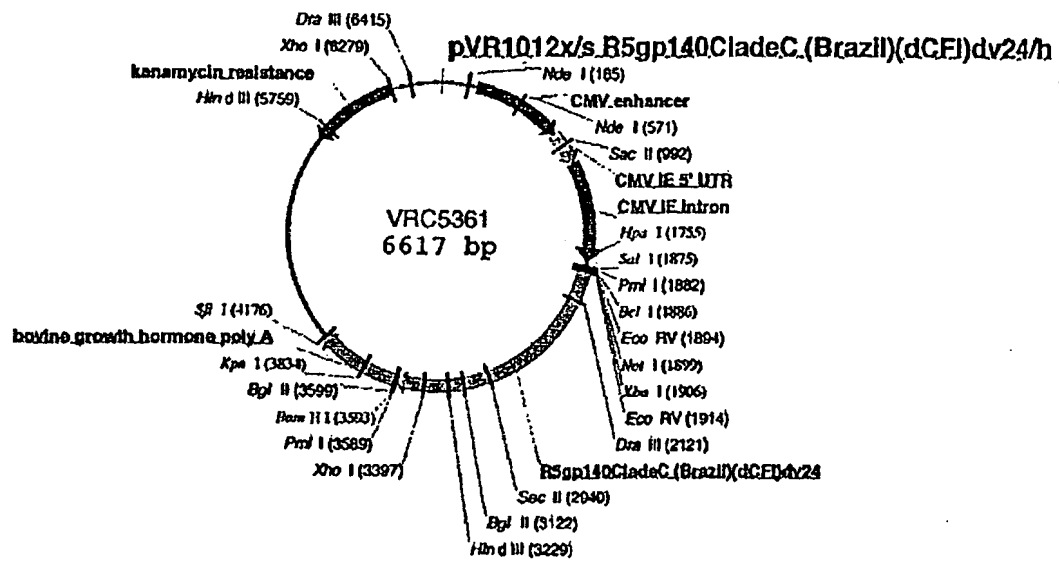


Figure 108

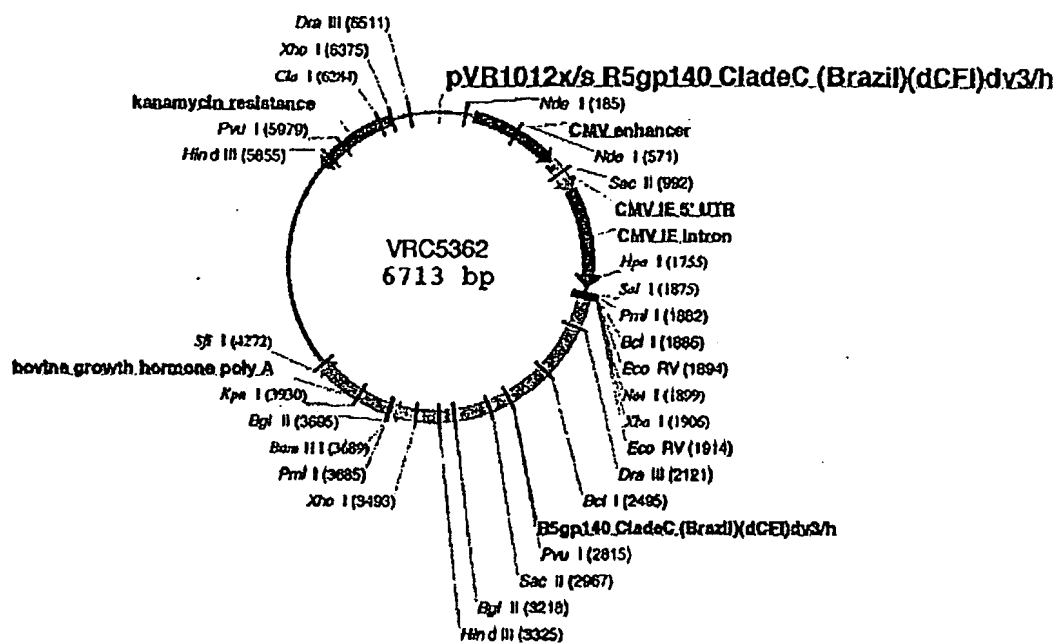


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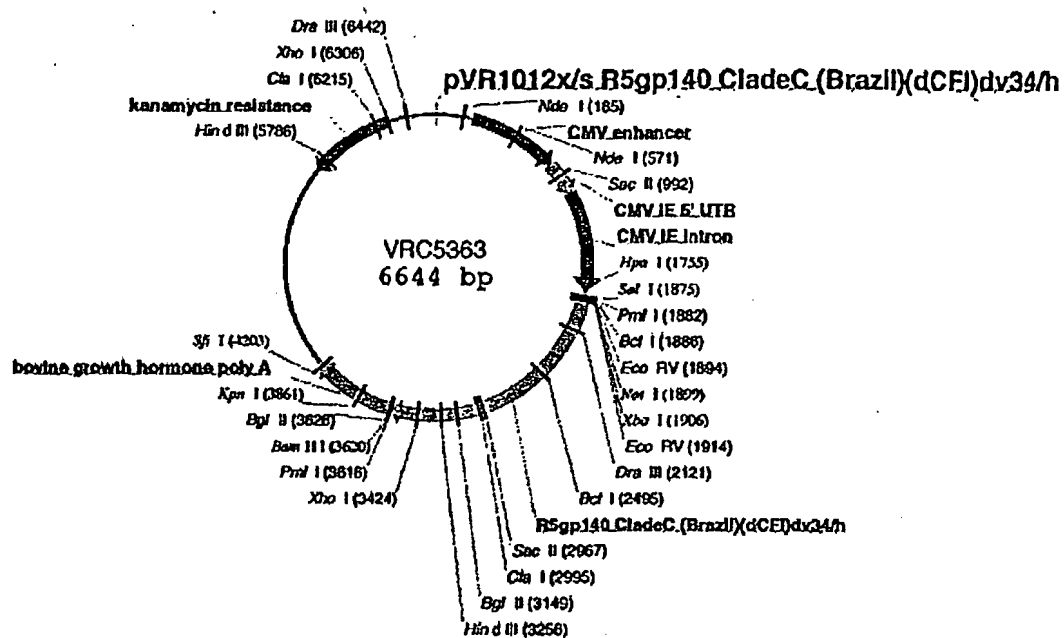


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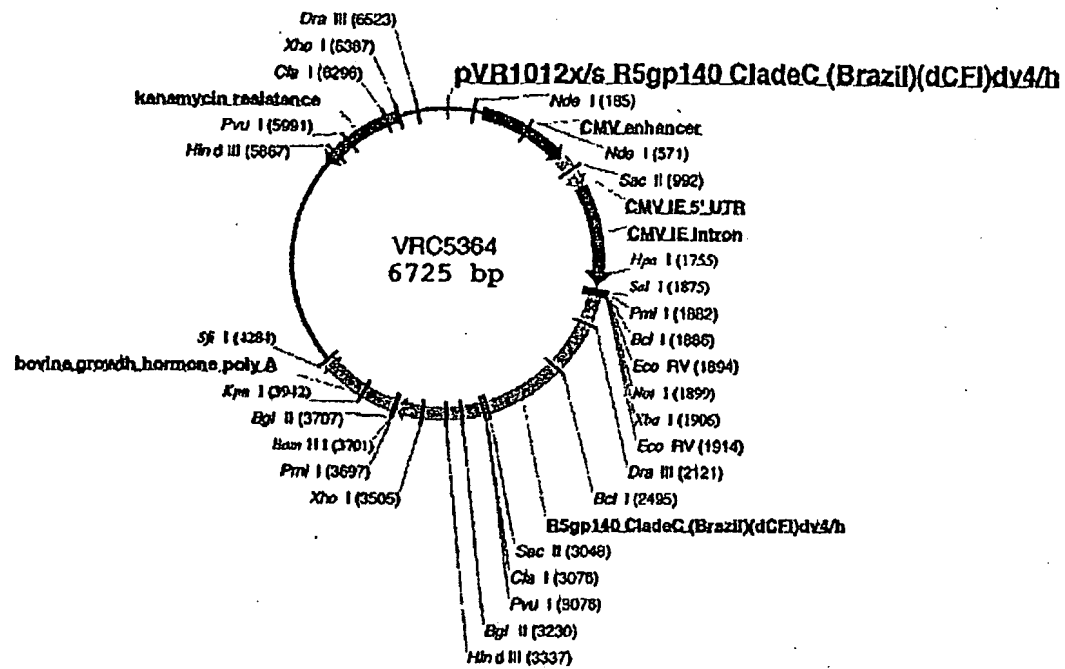


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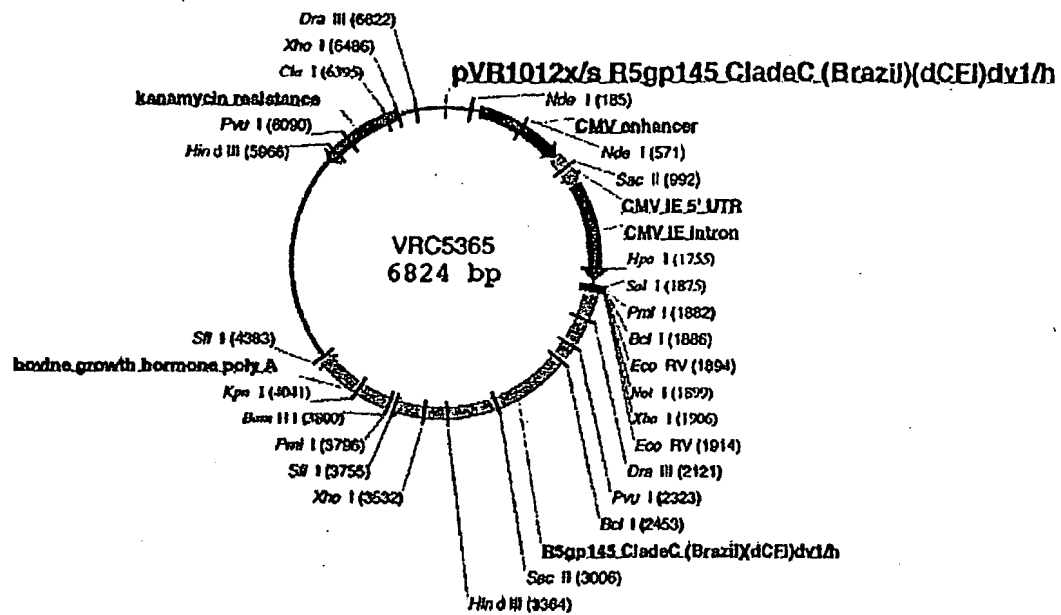


Figure 112

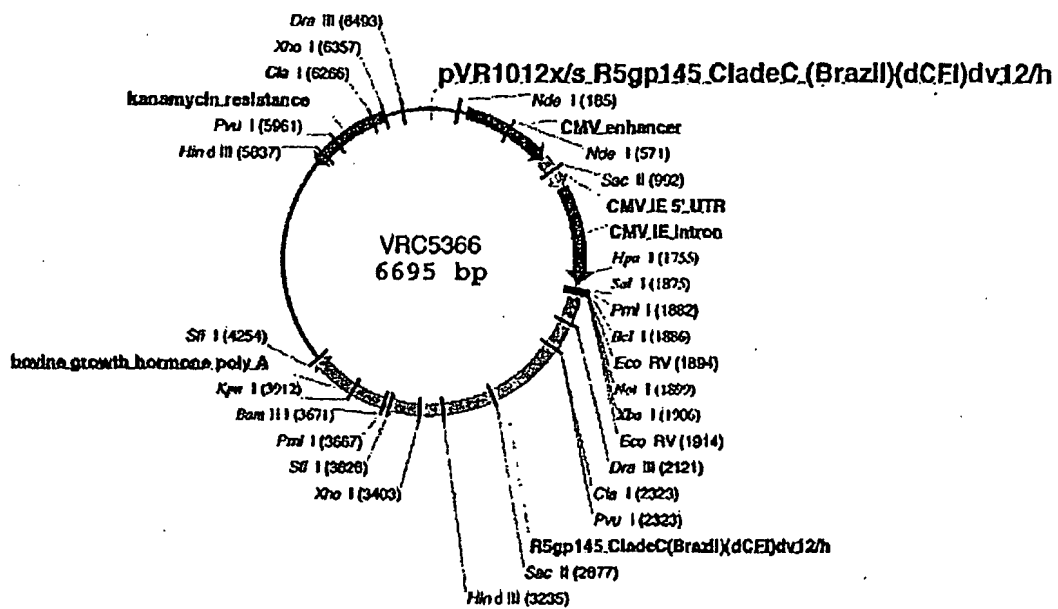


Figure 113

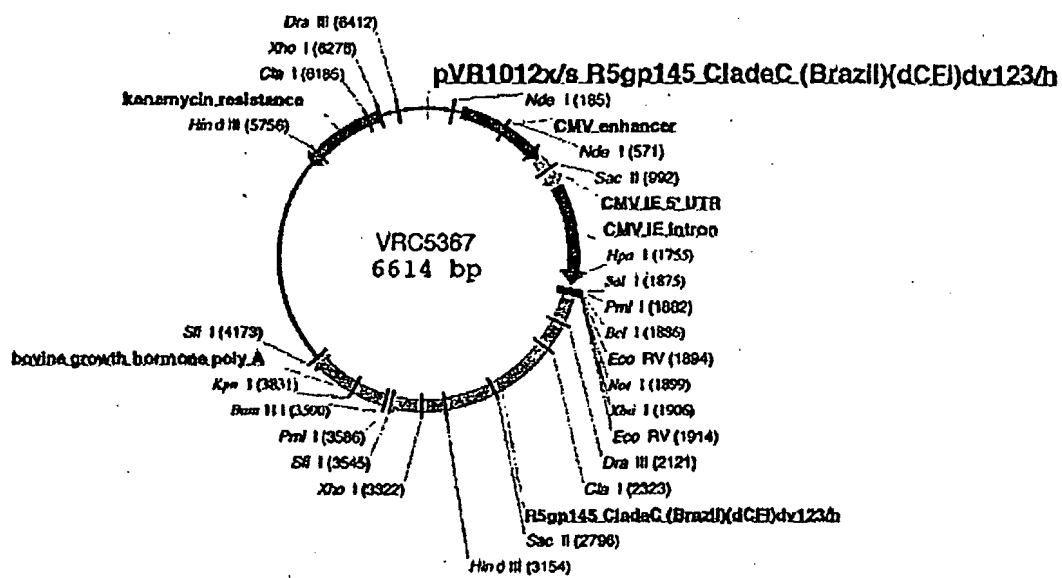


Figure 114

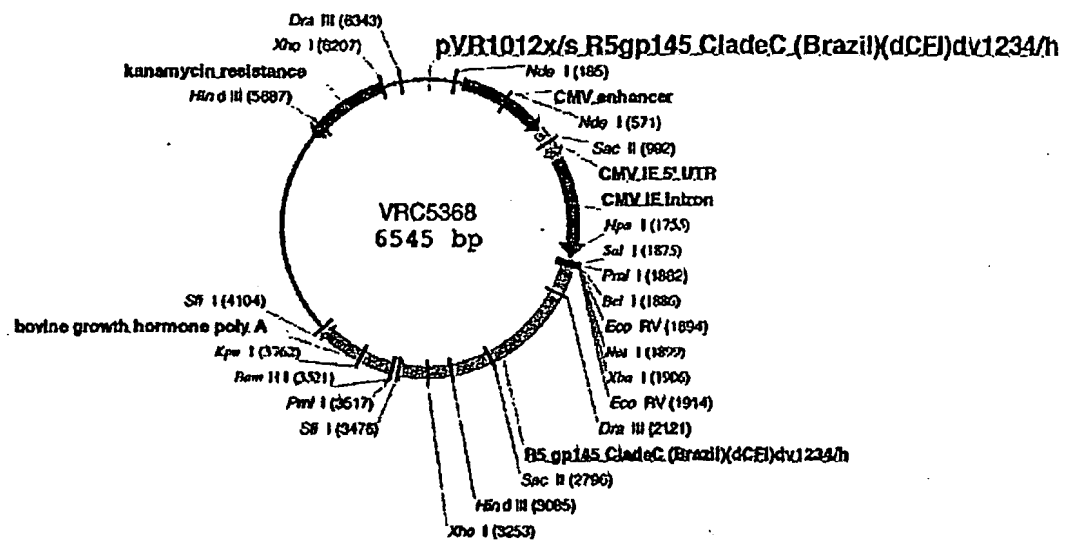


Figure 115

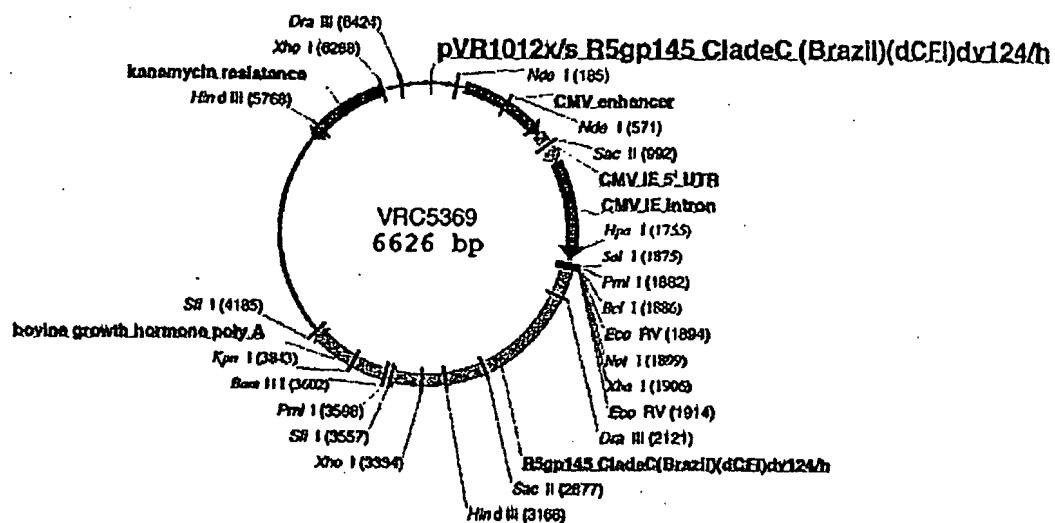


Figure 116

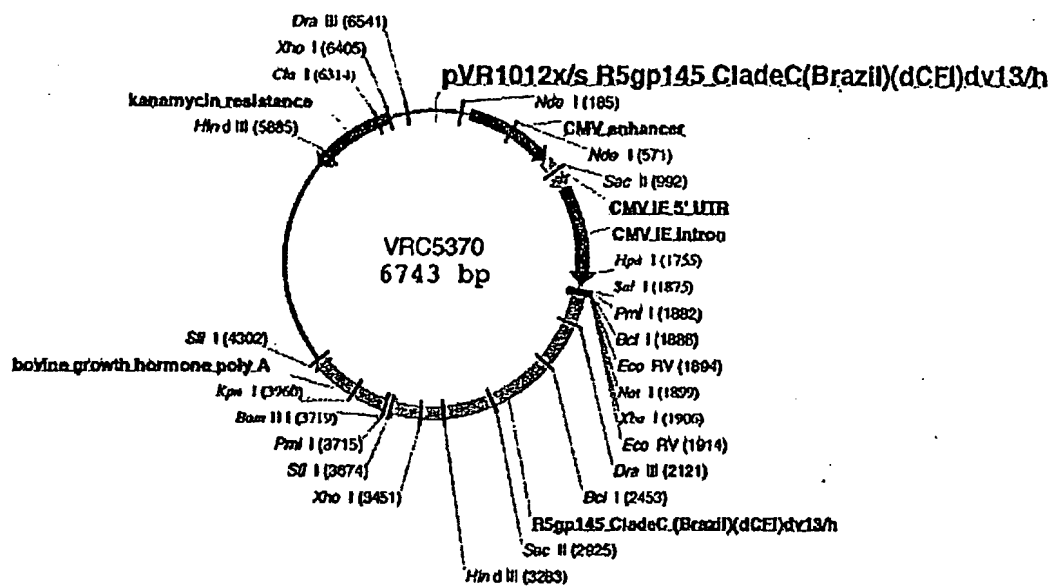


Figure 117

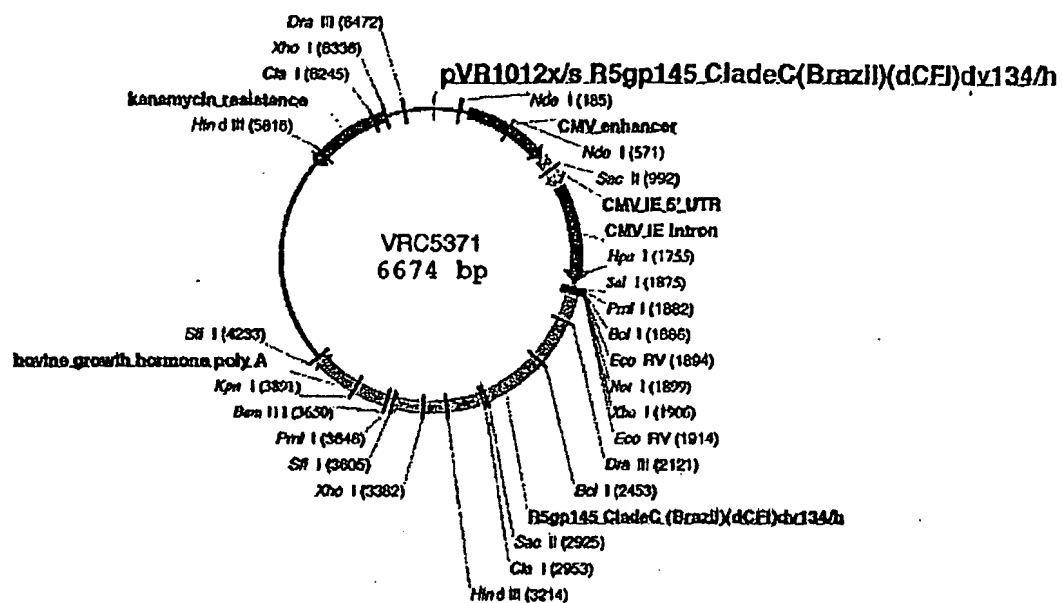


Figure 118

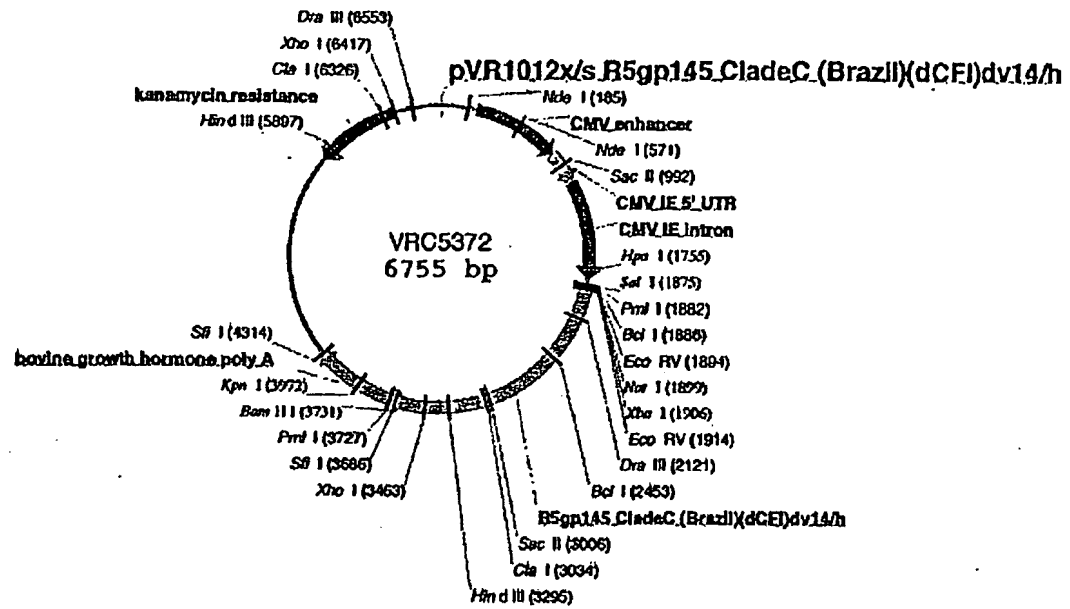


Figure 119

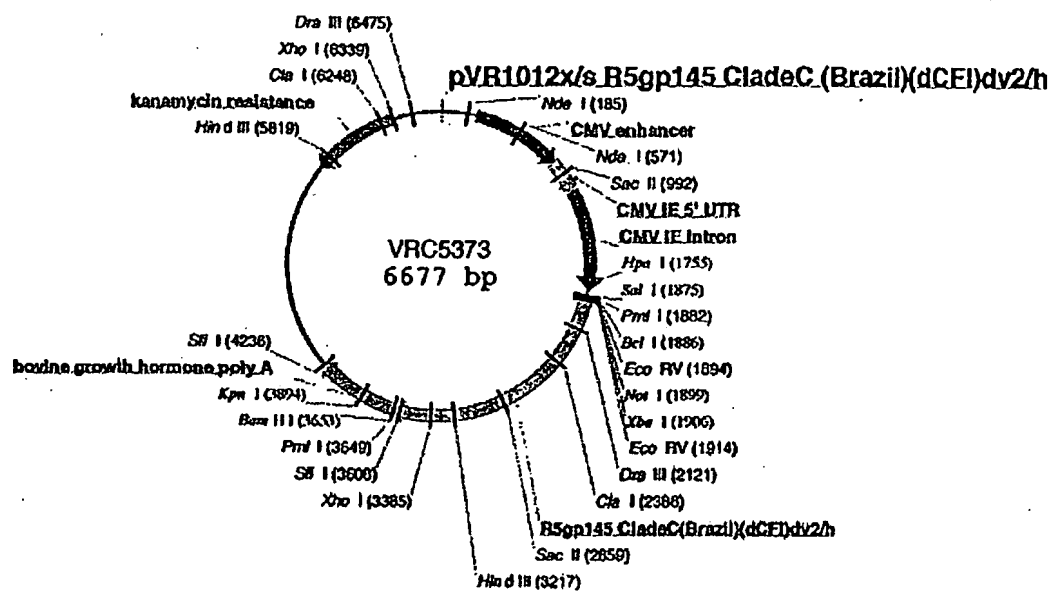


Figure 120

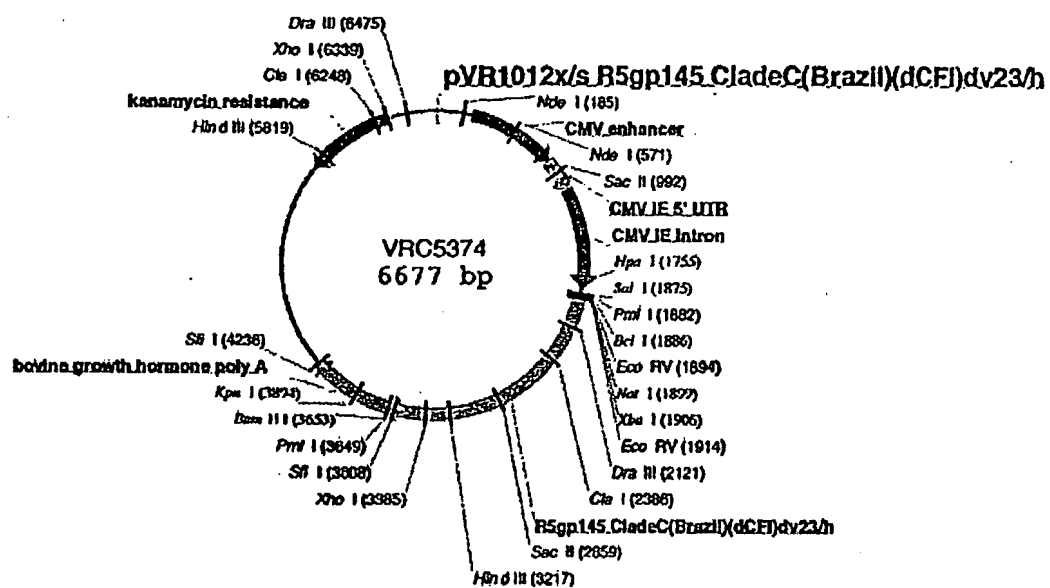


Figure 121

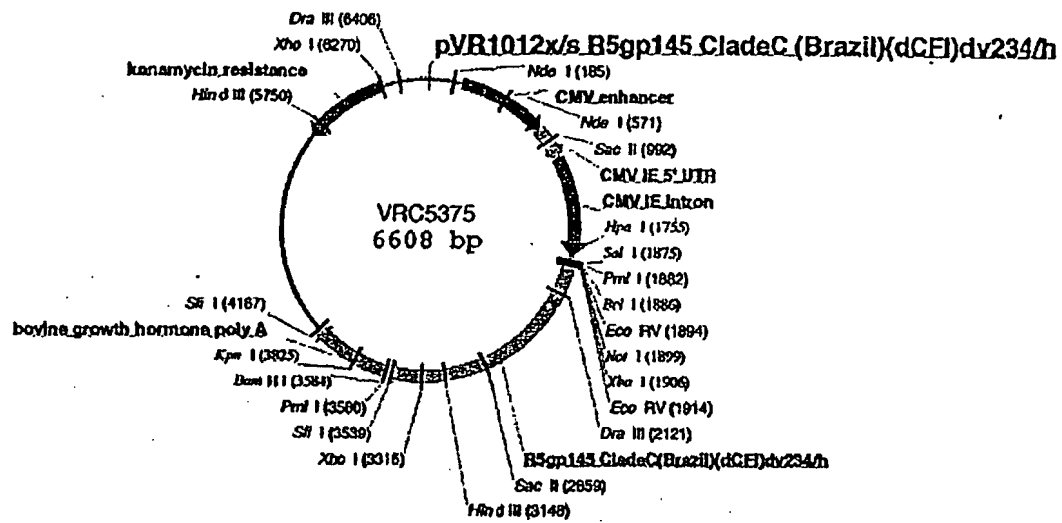


Figure 122

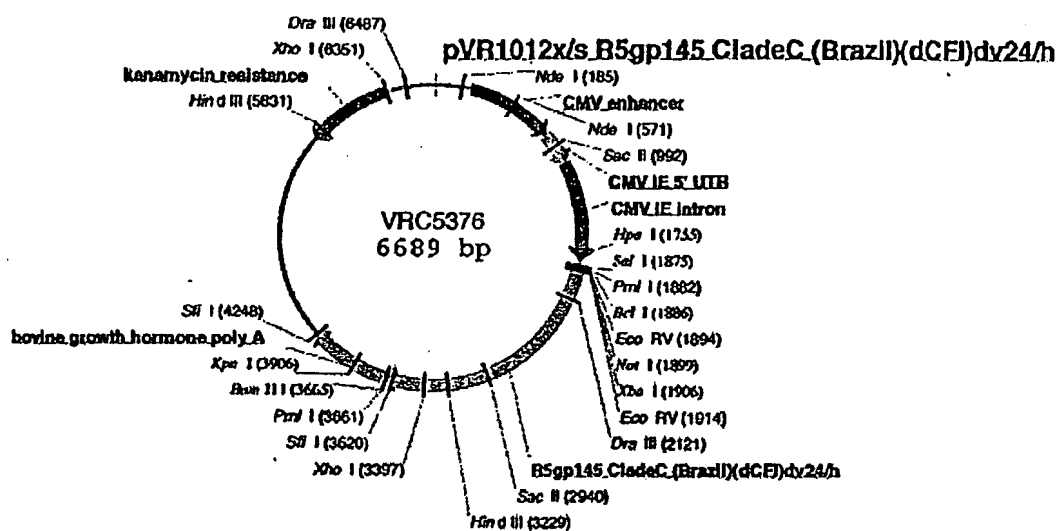


Figure 123

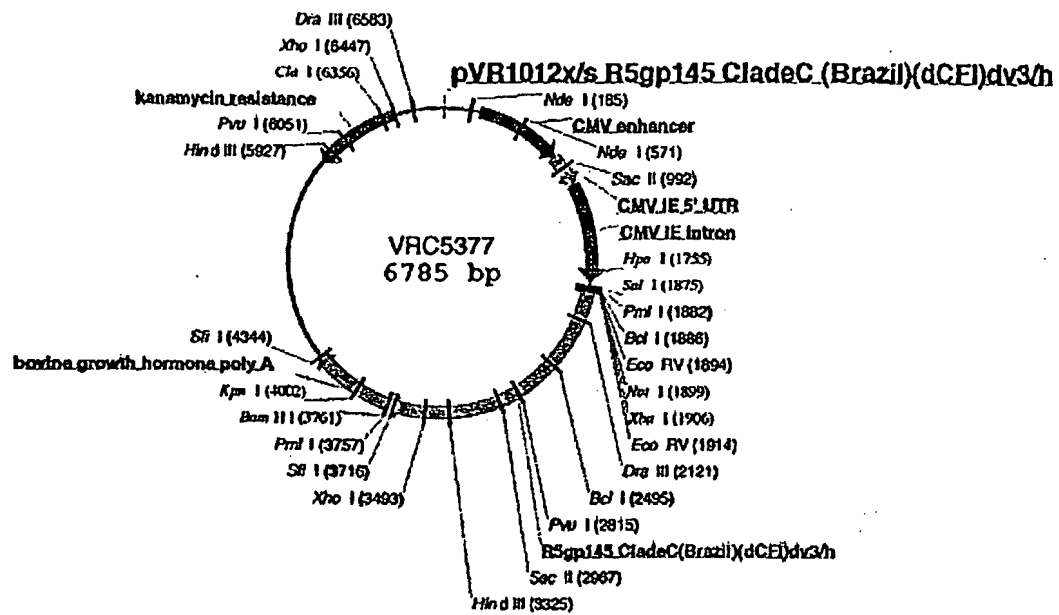


Figure 124

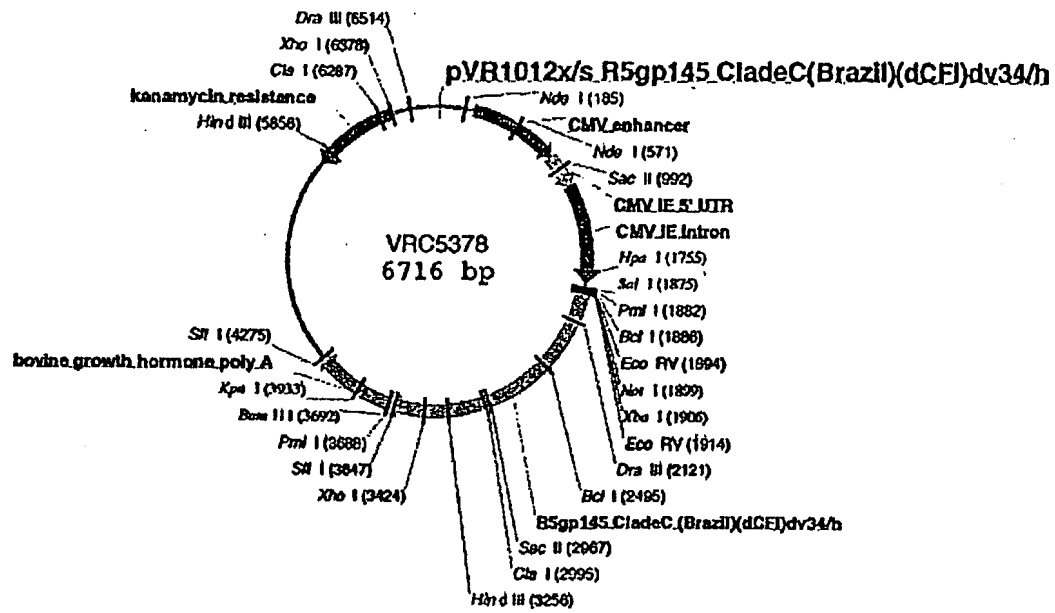


Figure 125

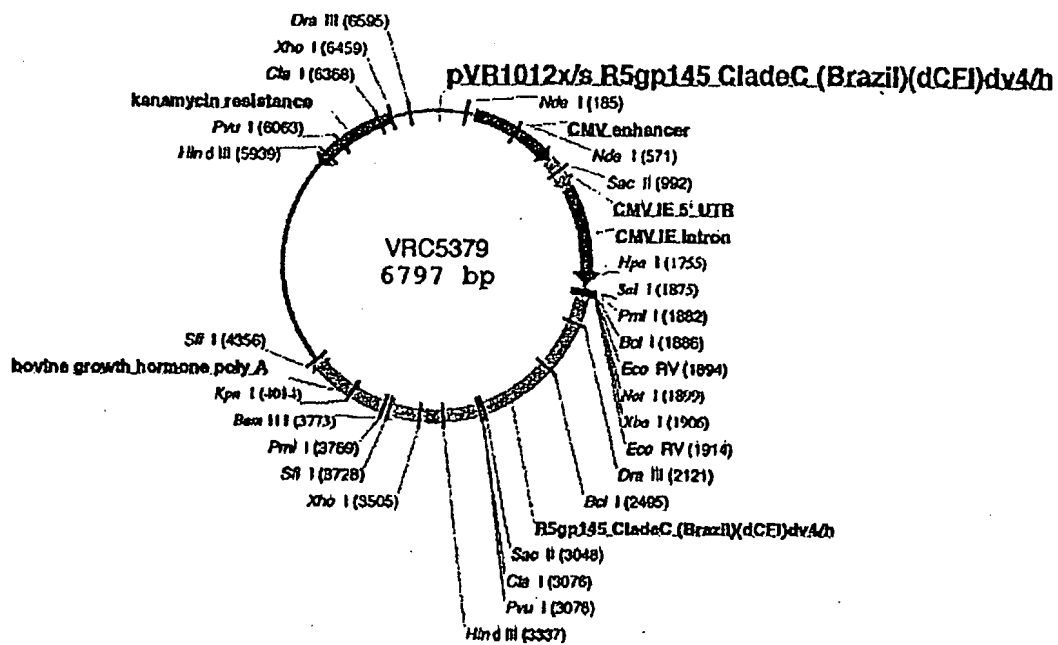


Figure 126

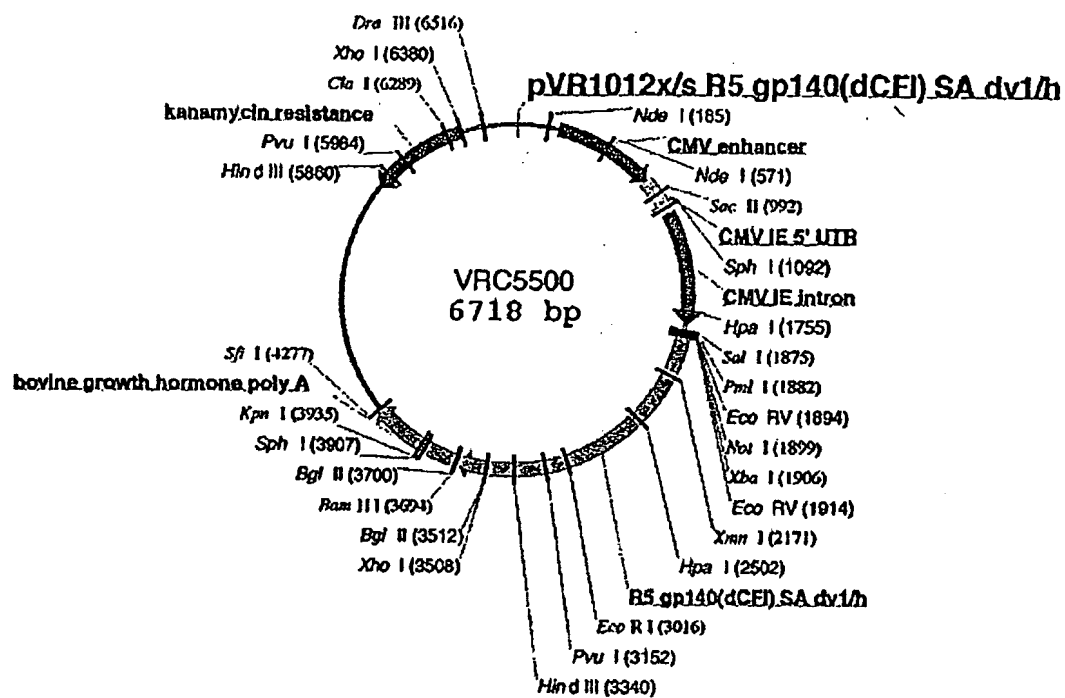


Figure 127

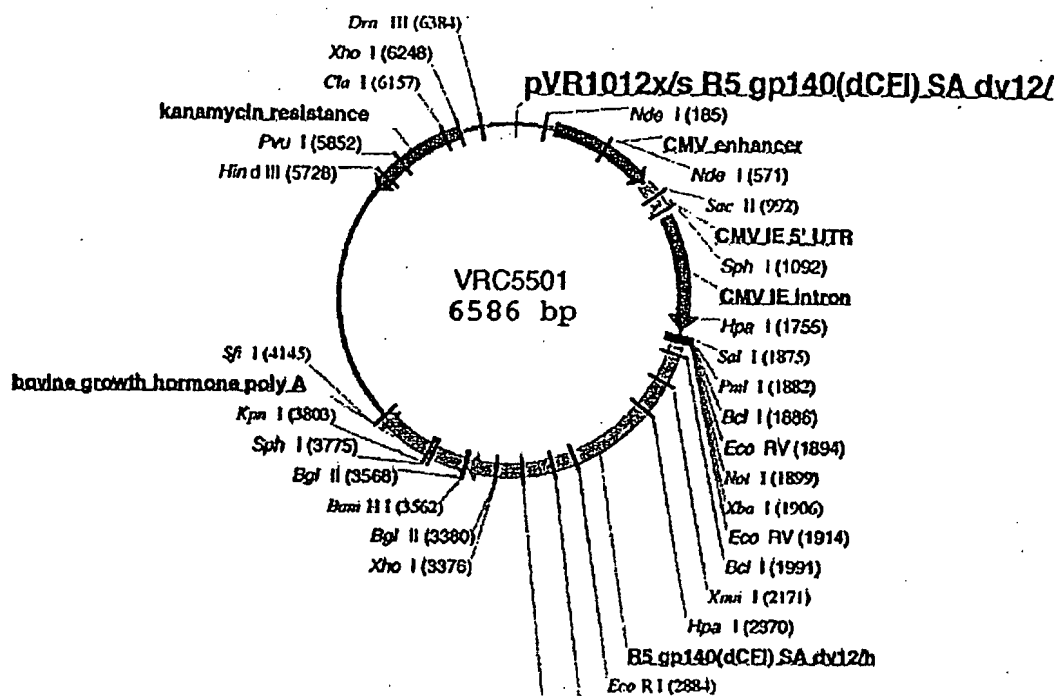


Figure 128

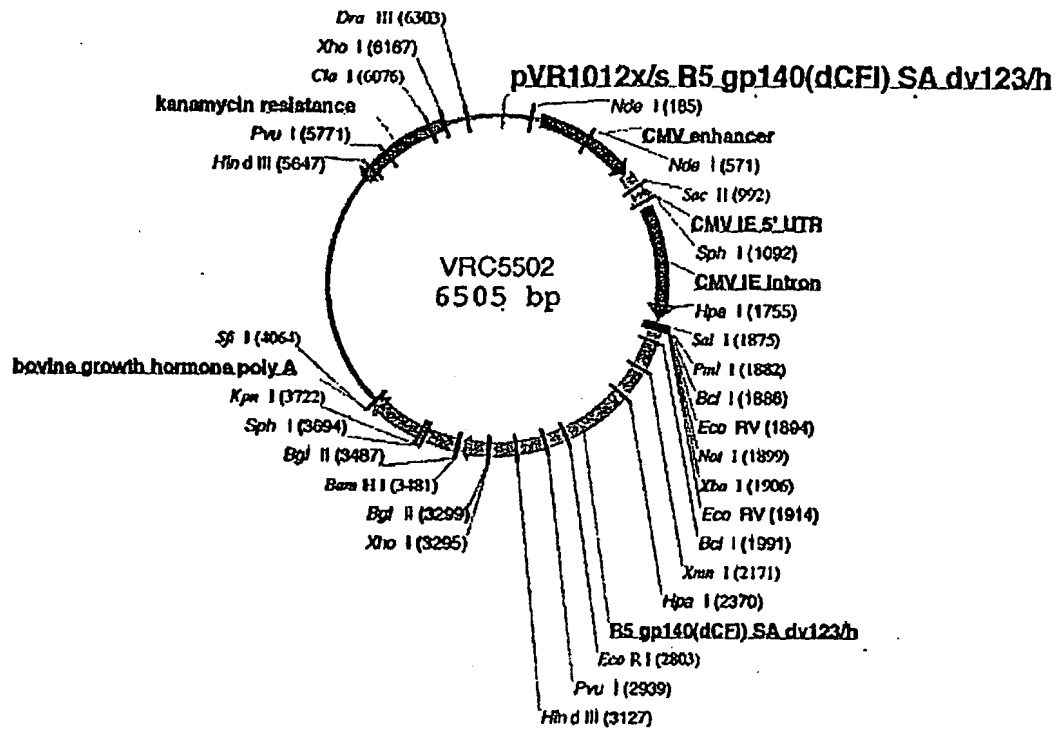


Figure 129

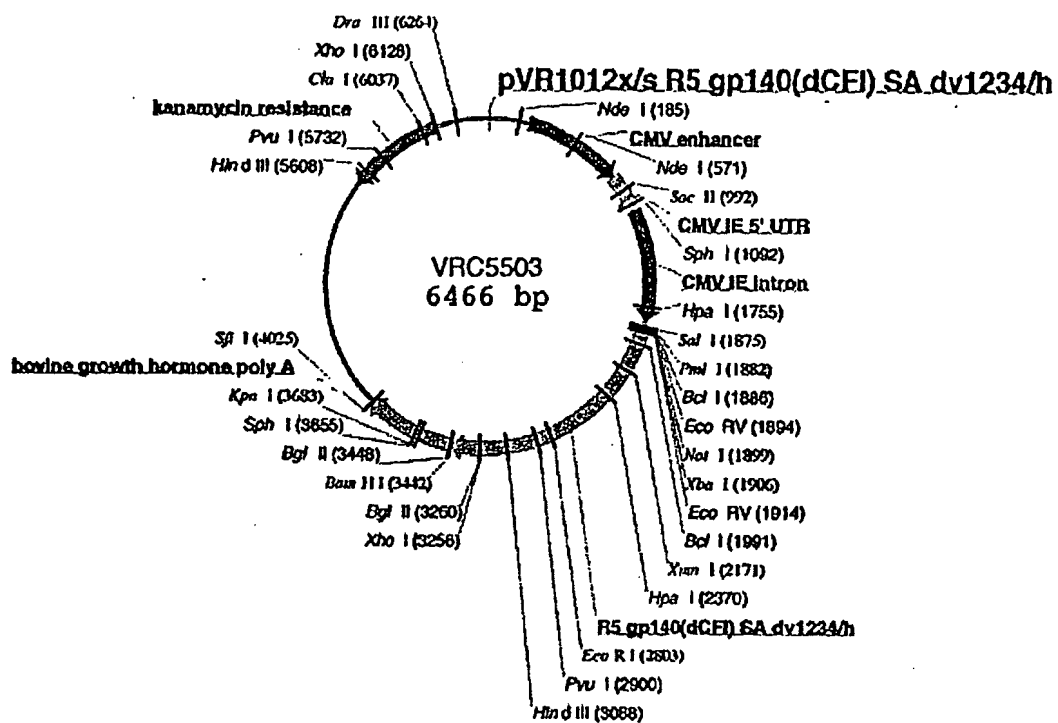


Figure 130

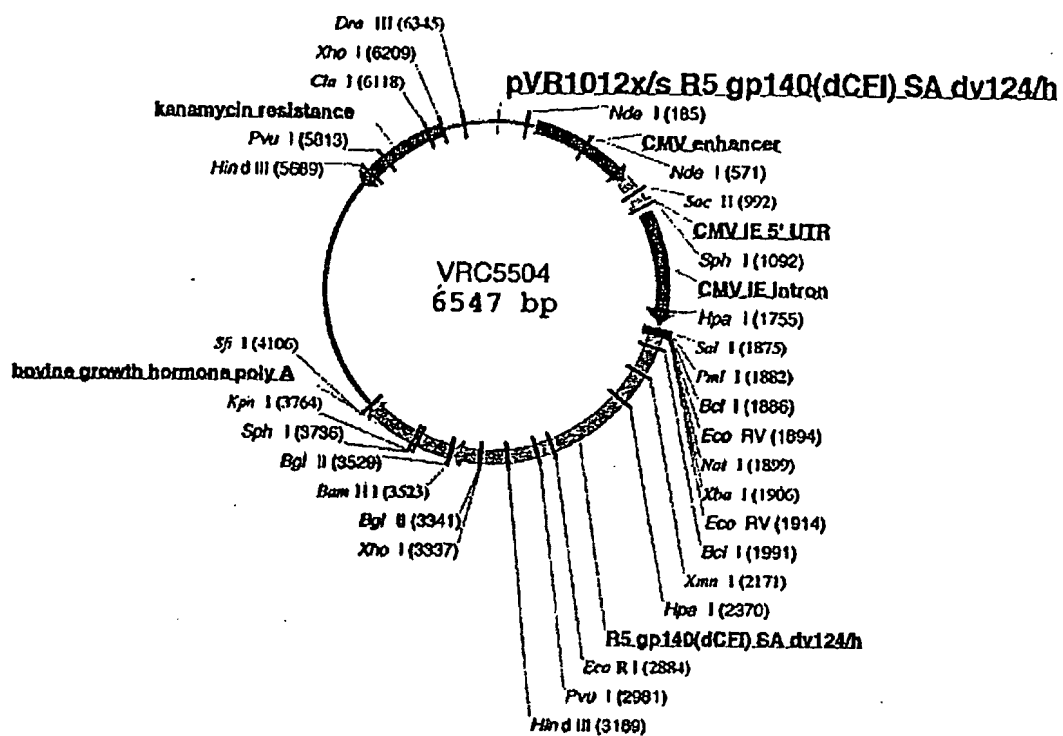


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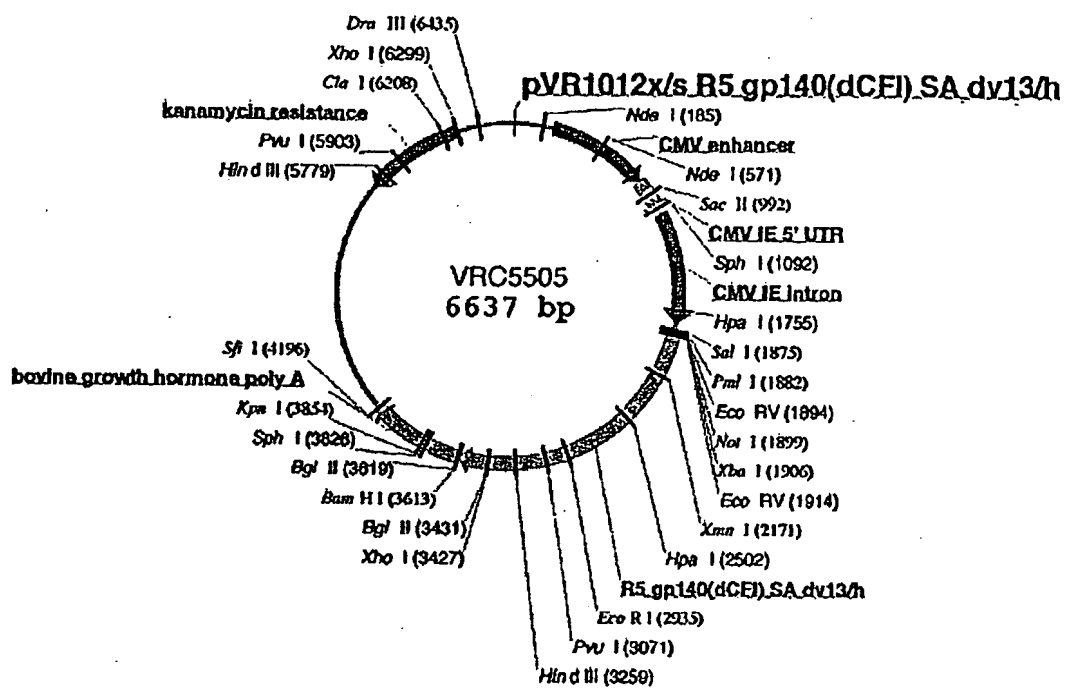


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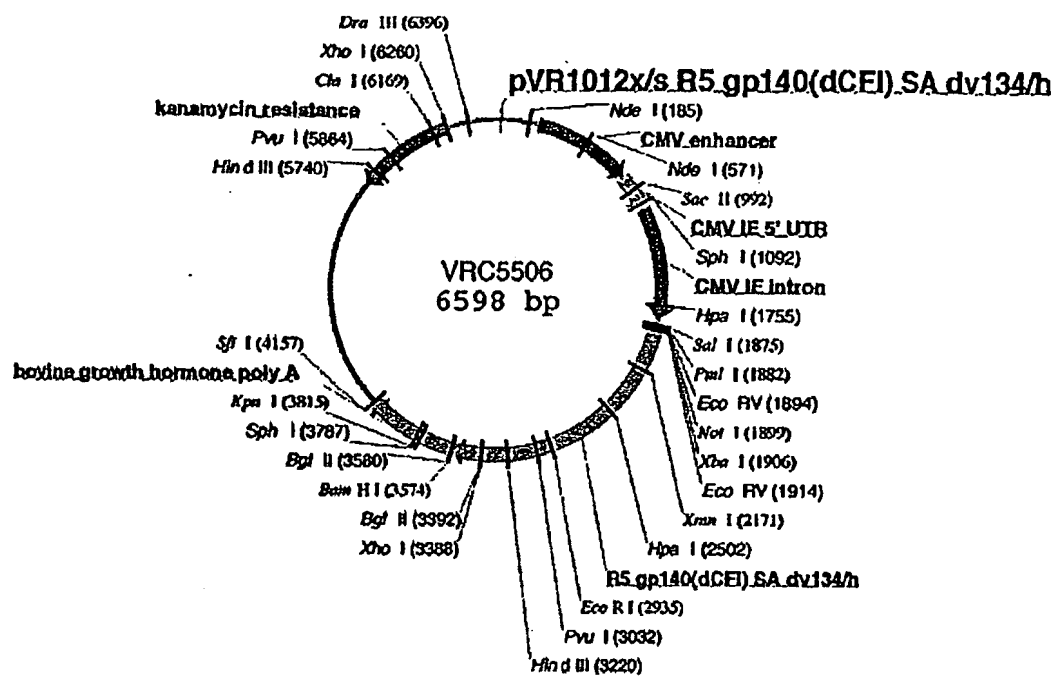


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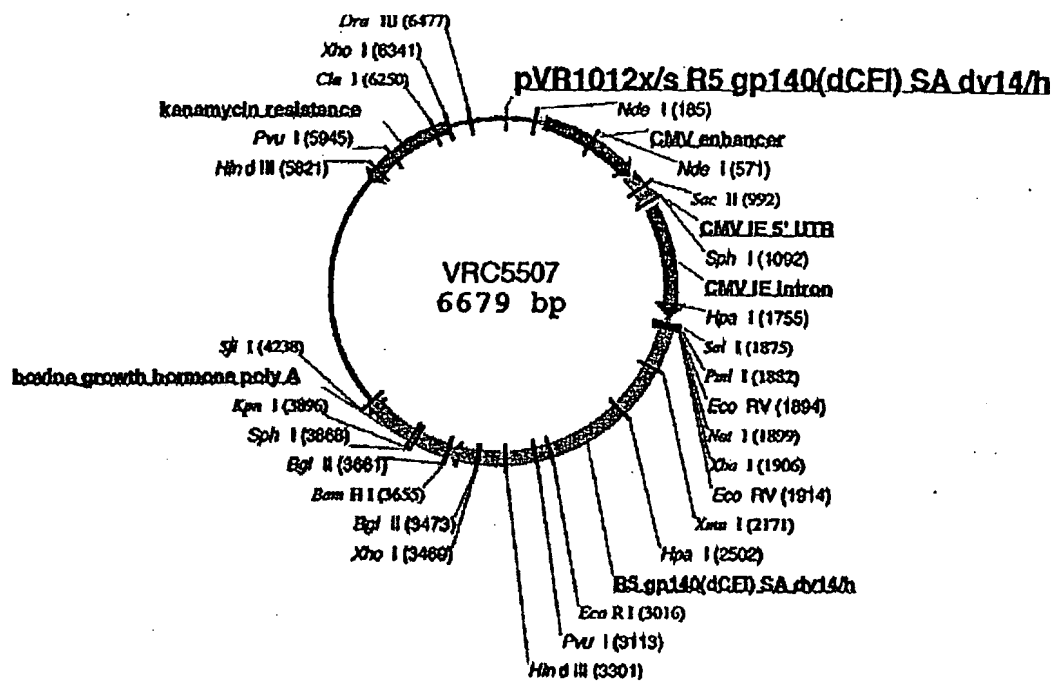


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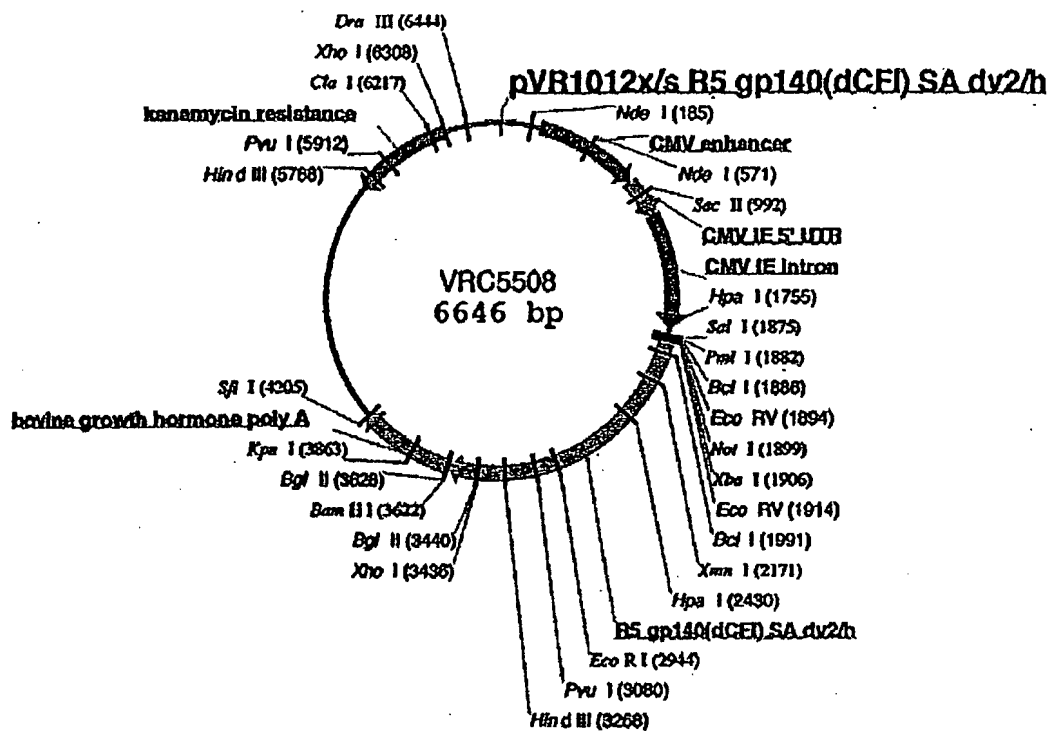


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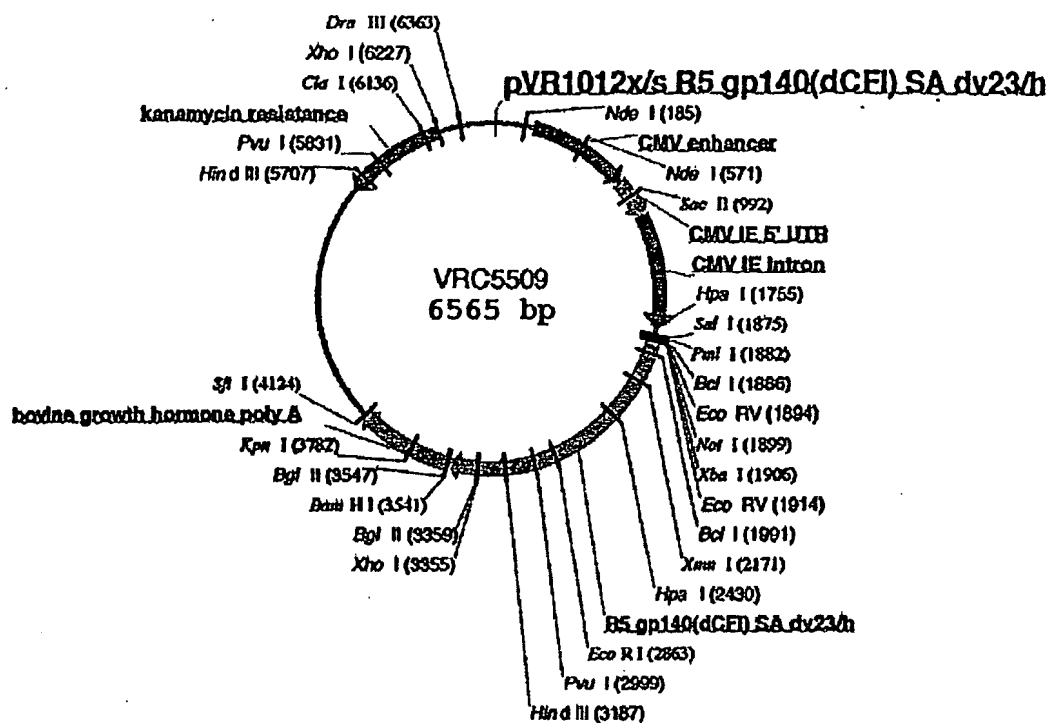


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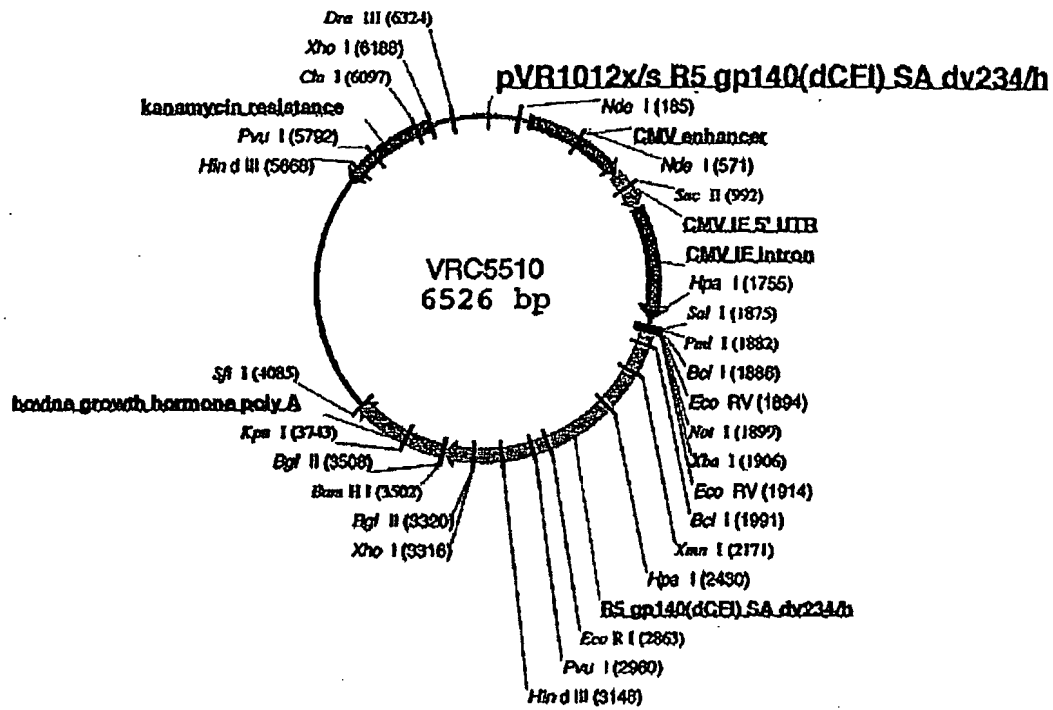


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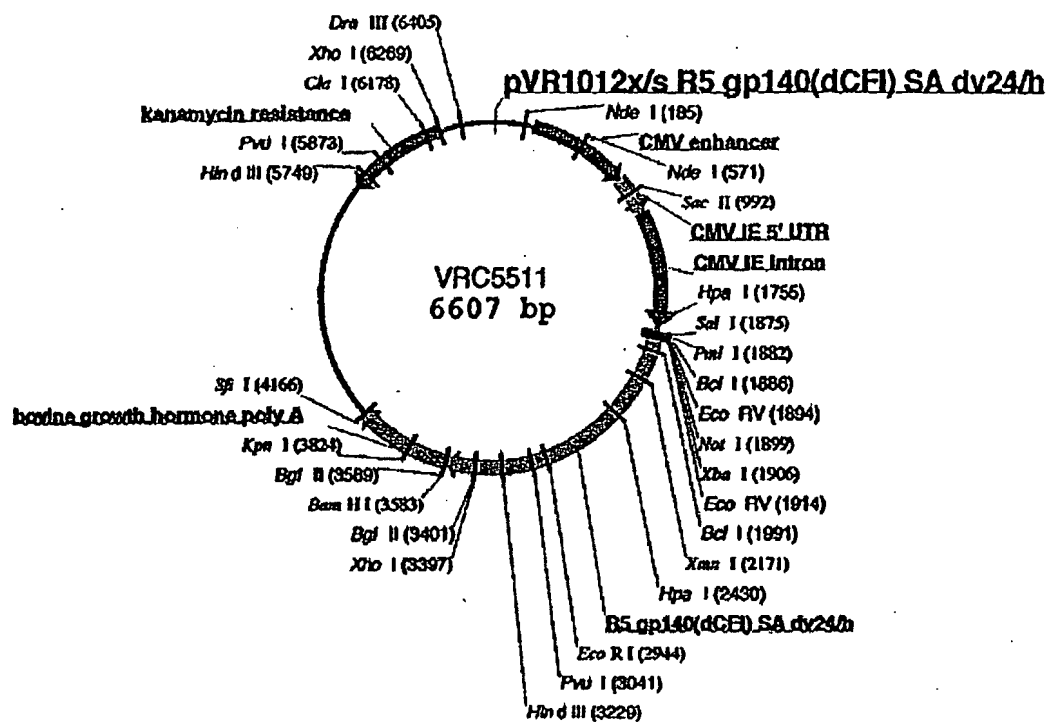
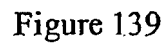


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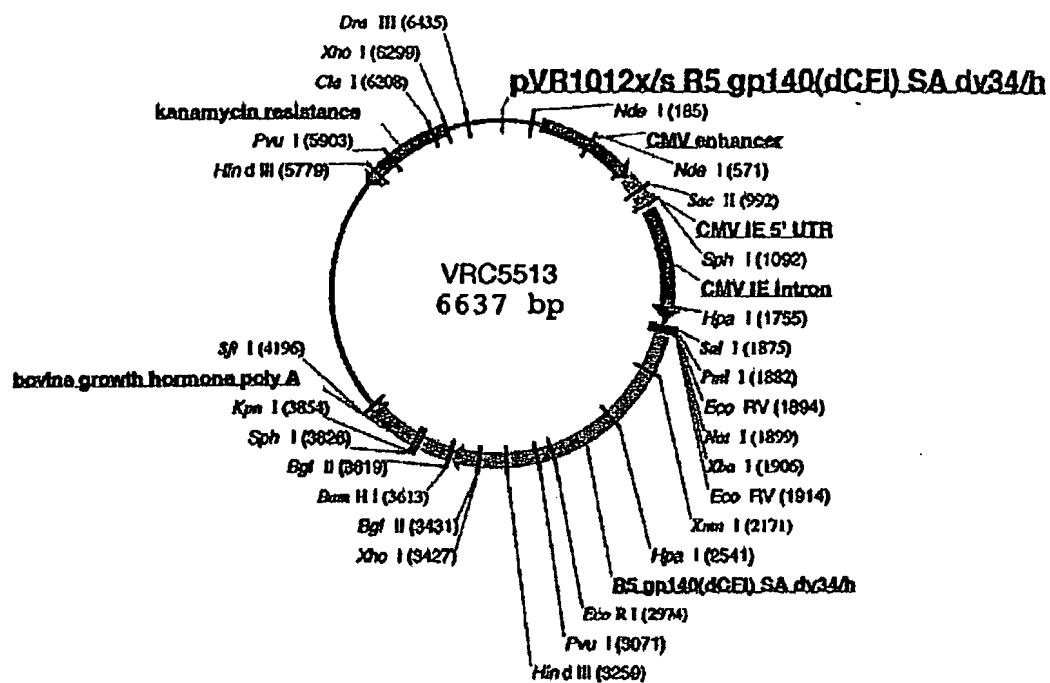


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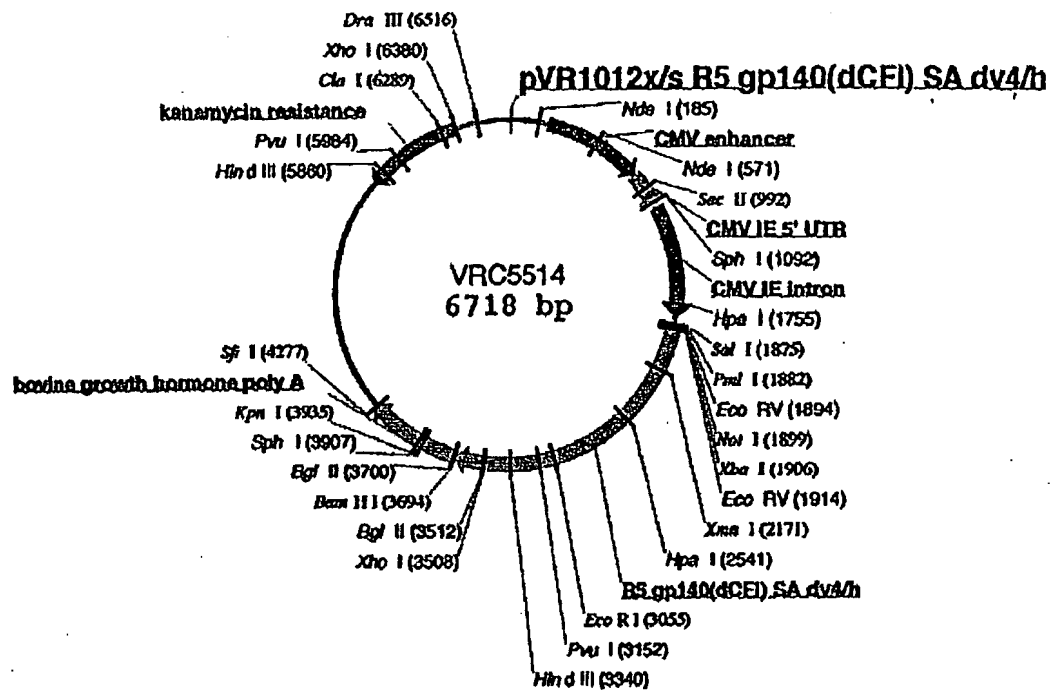


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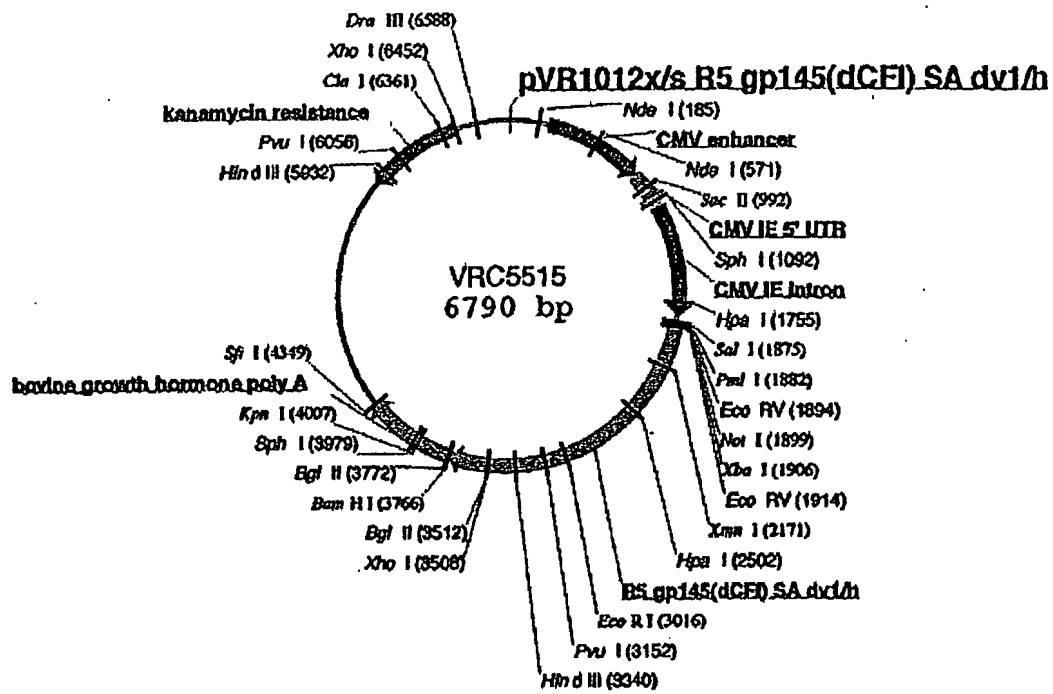


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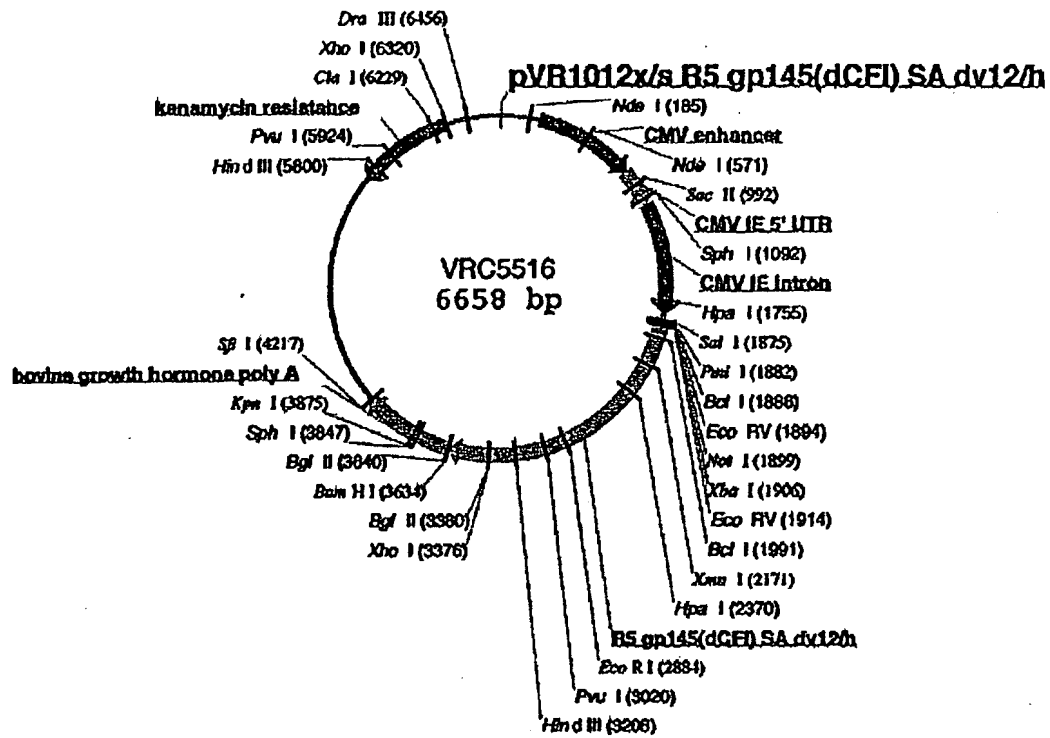


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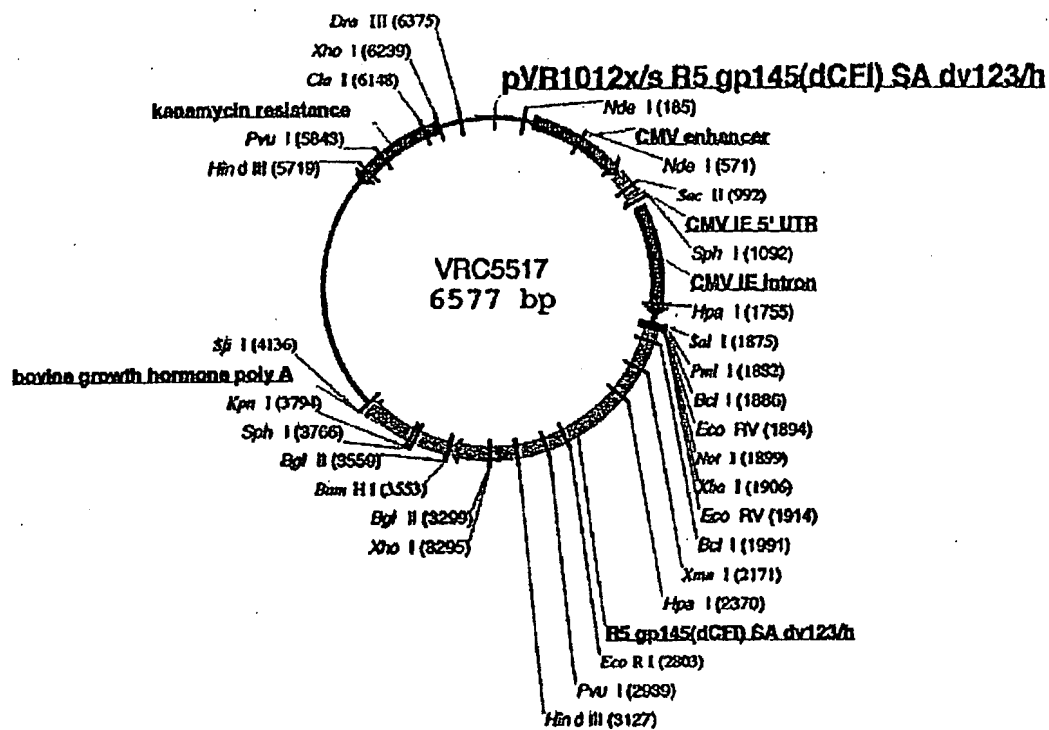


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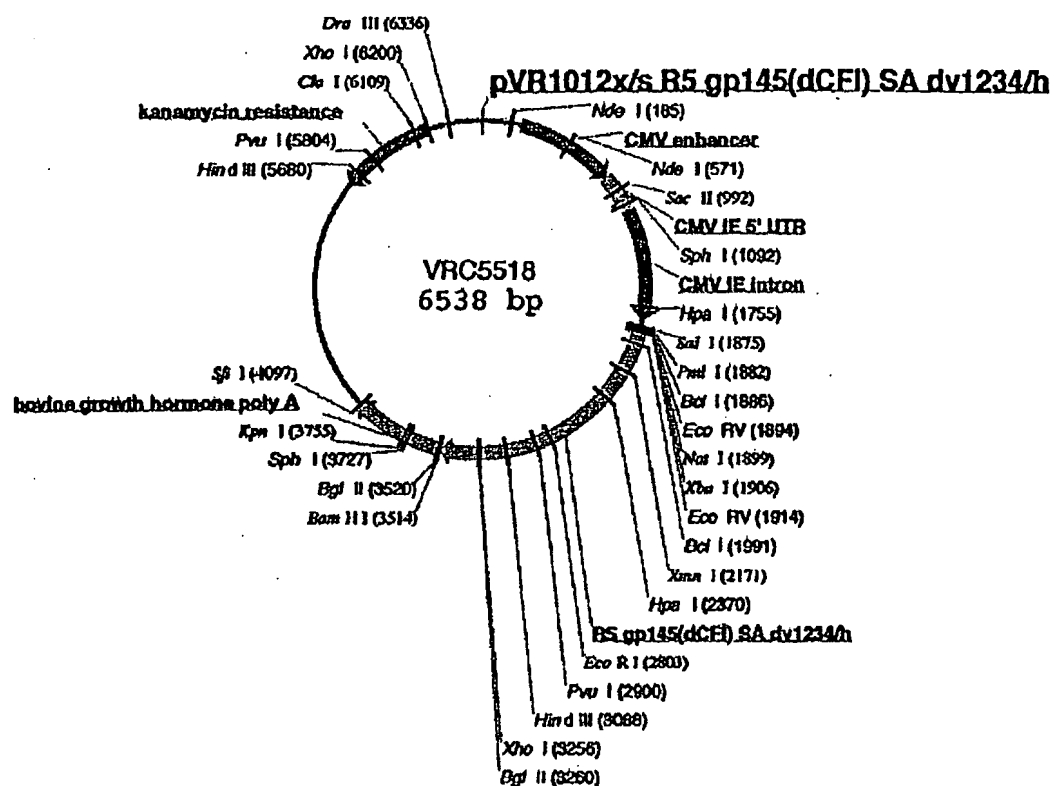


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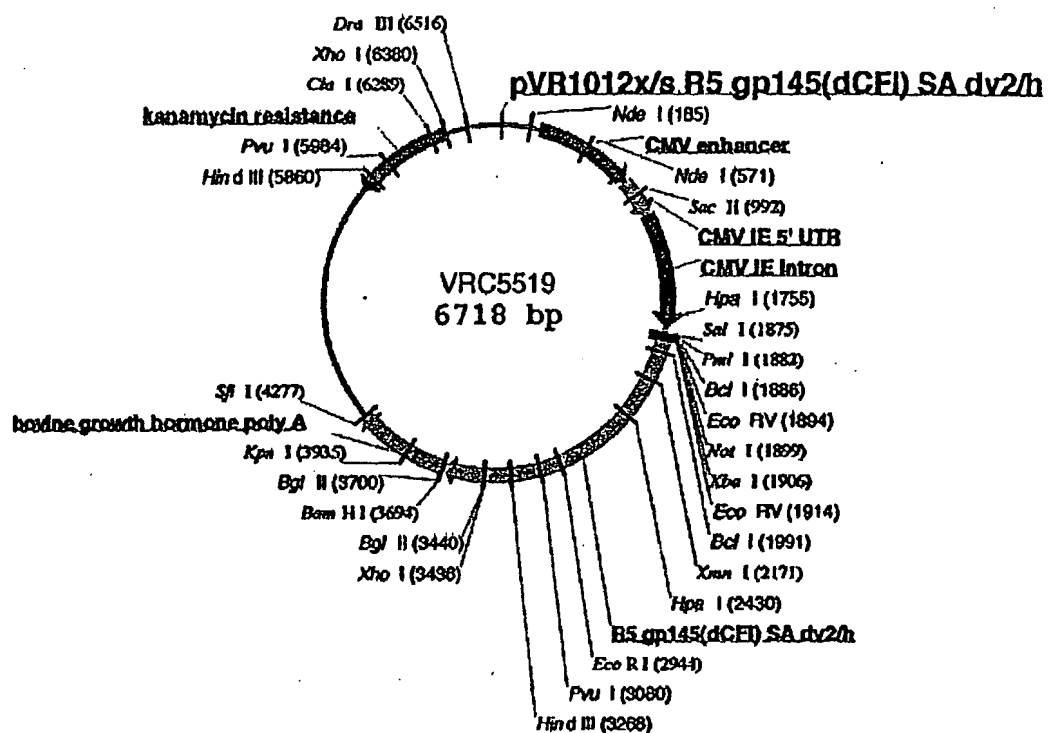


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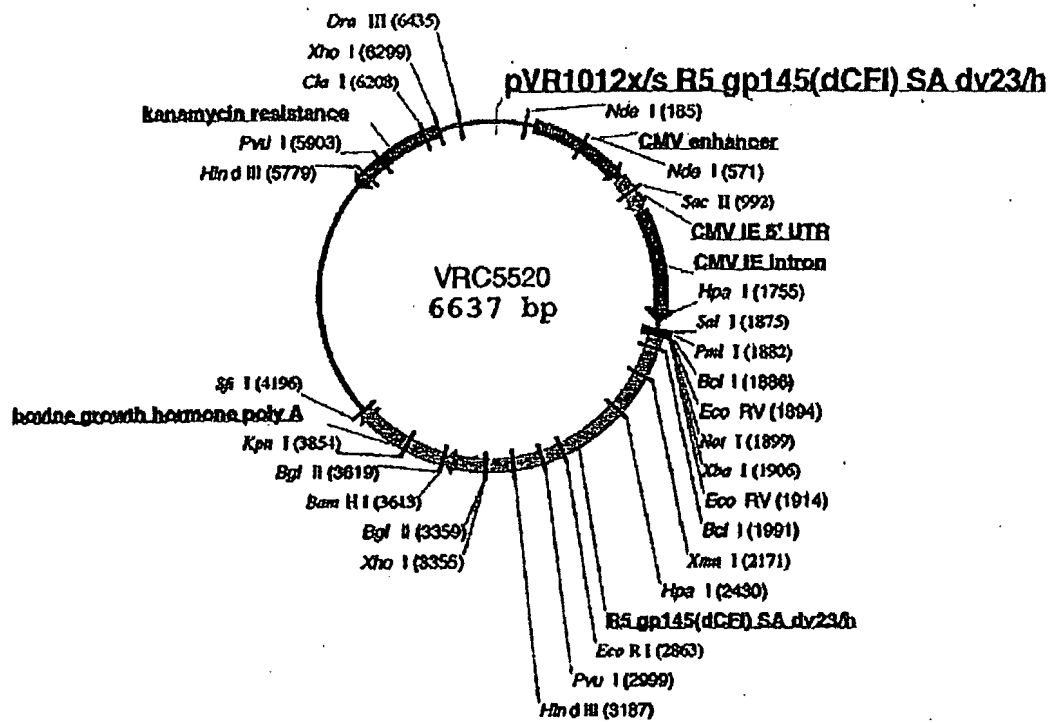


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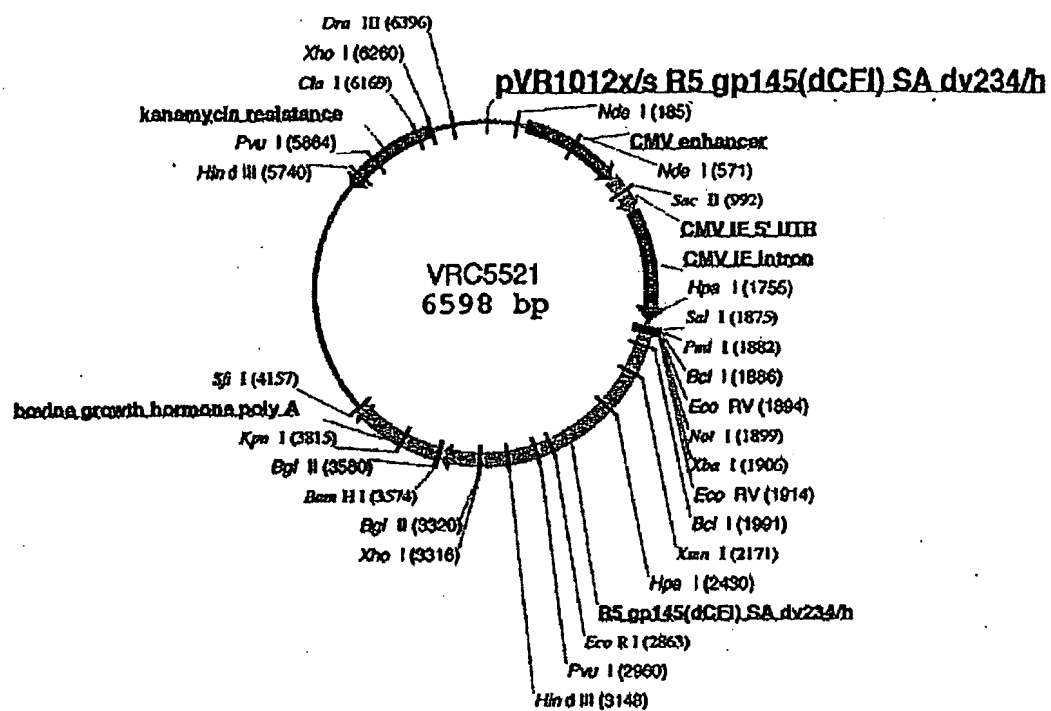


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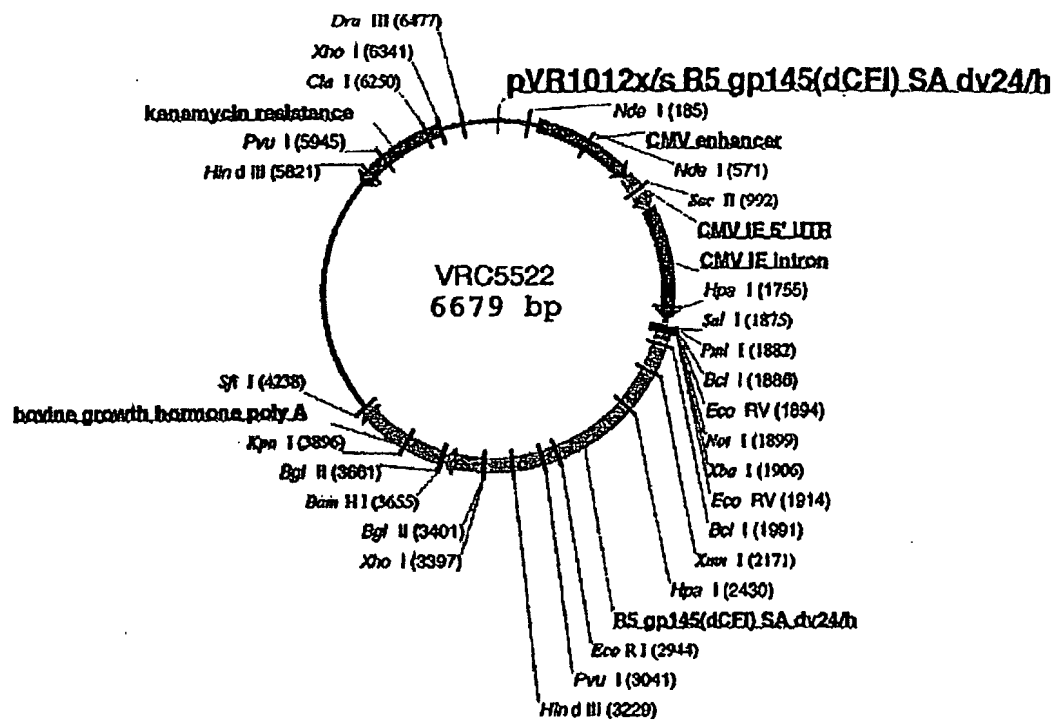


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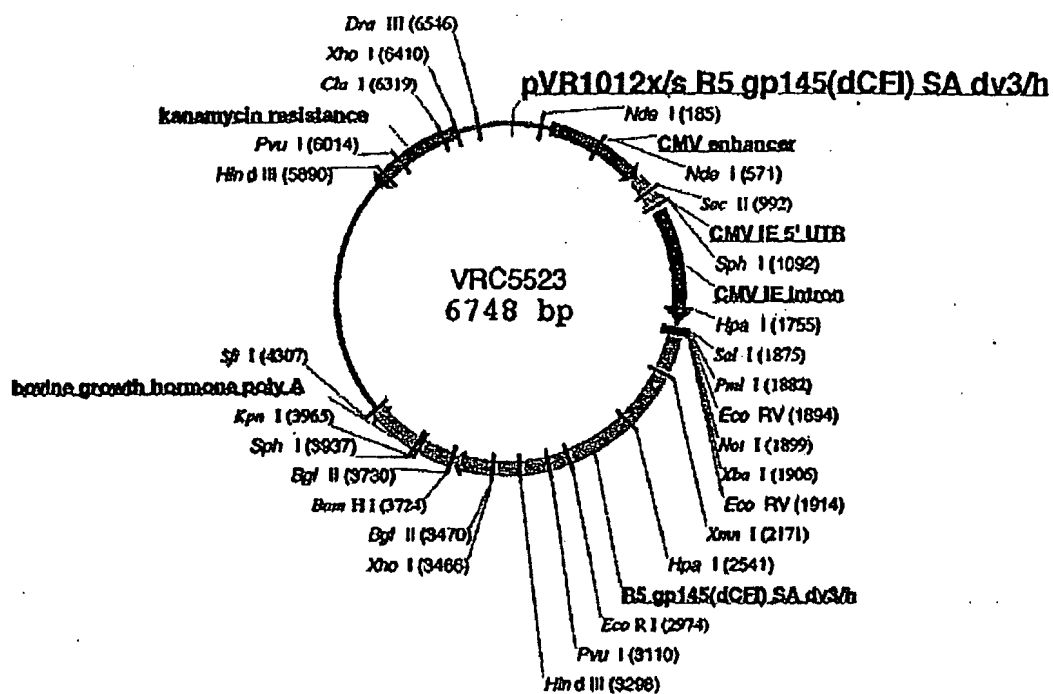


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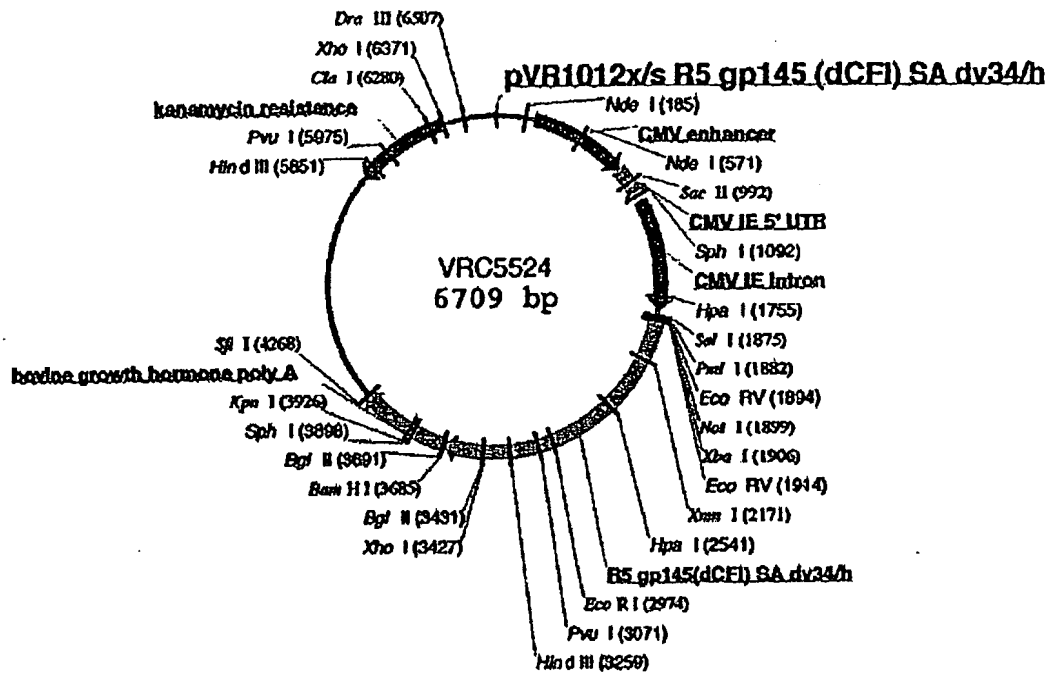


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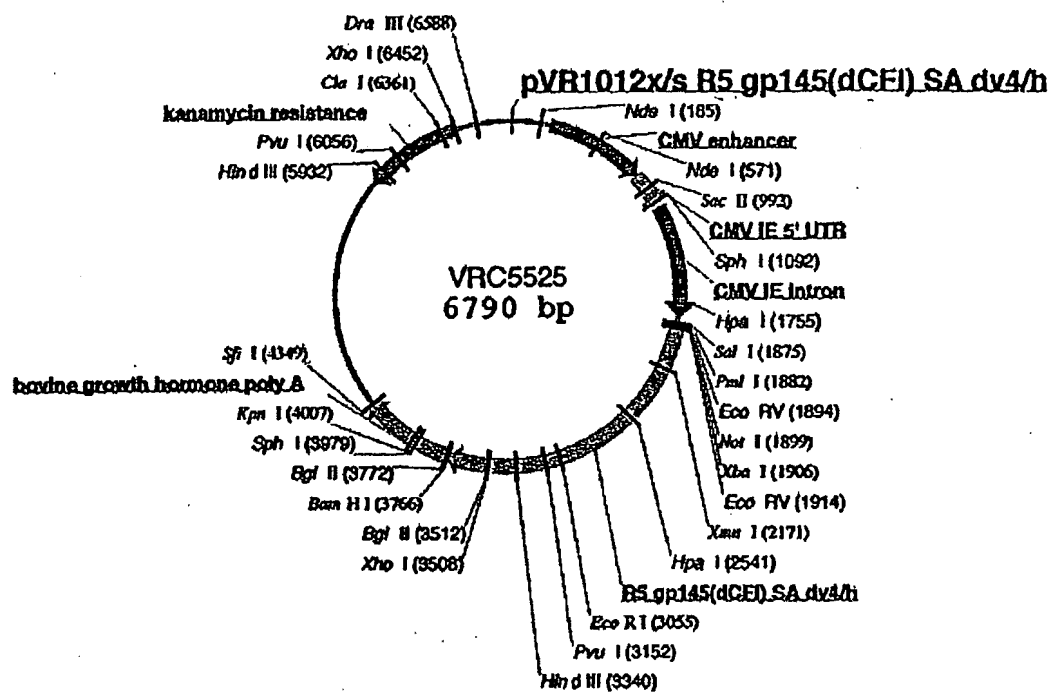


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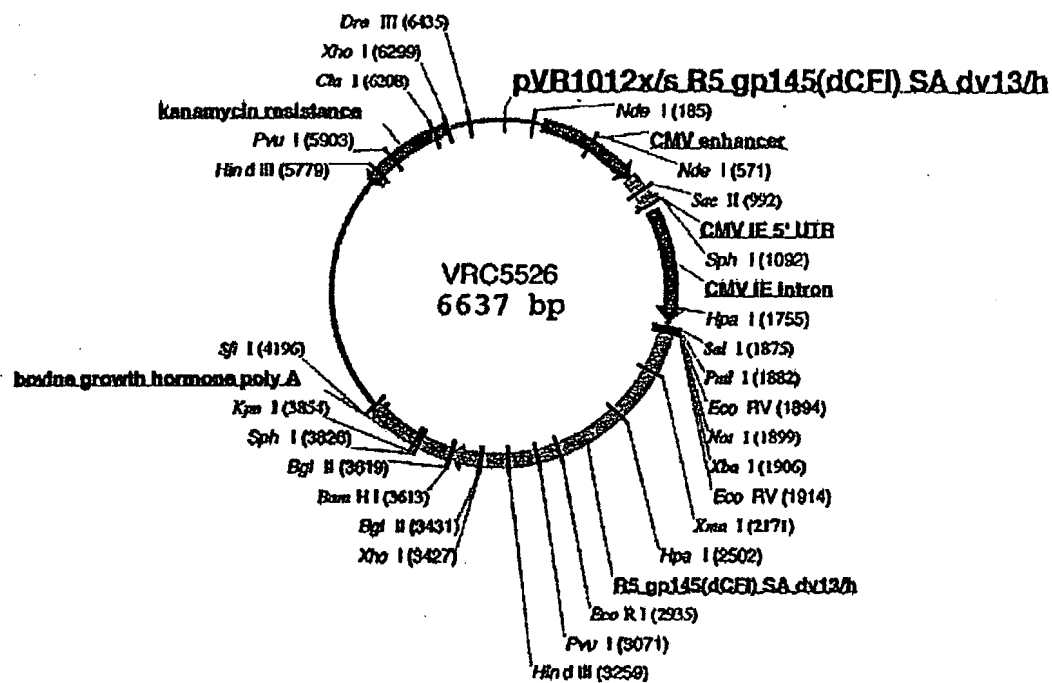


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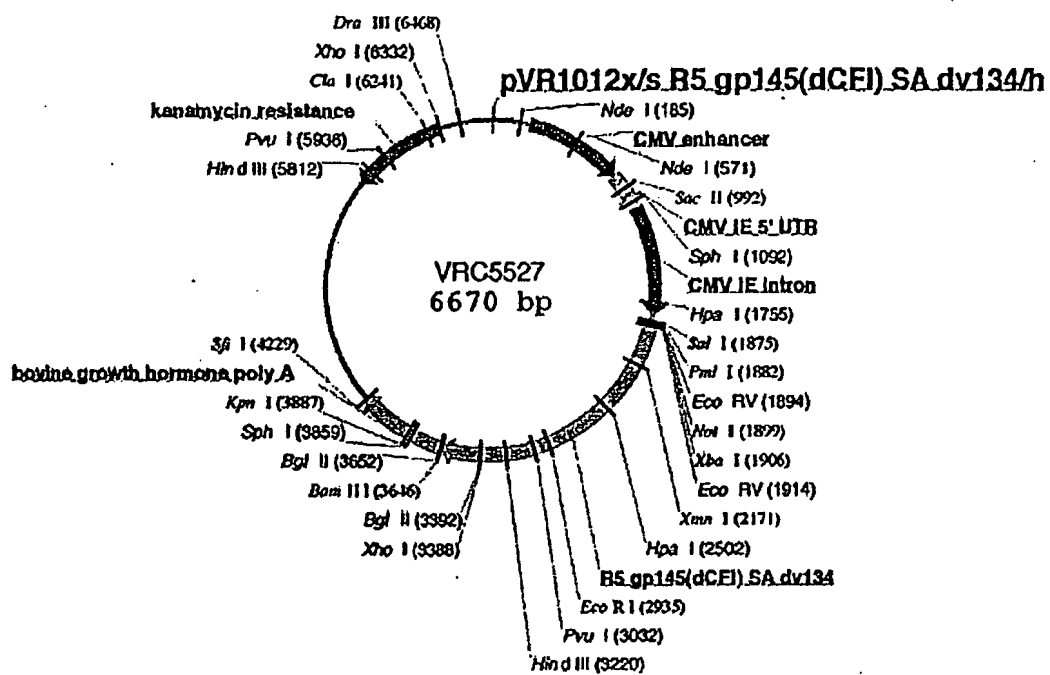


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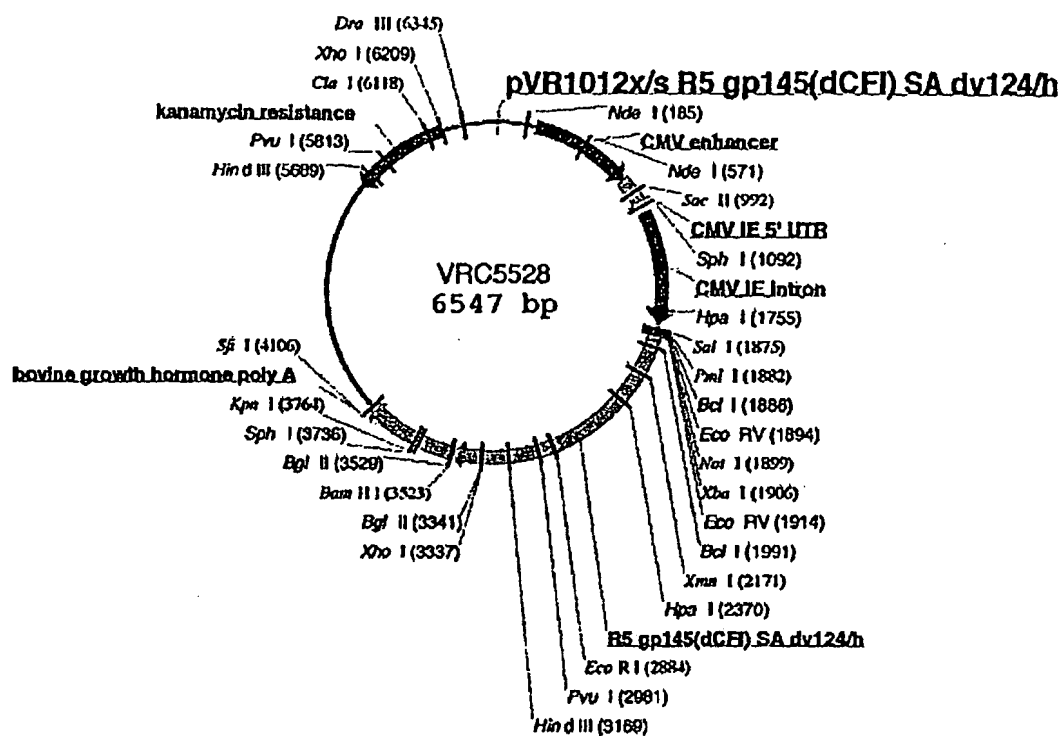


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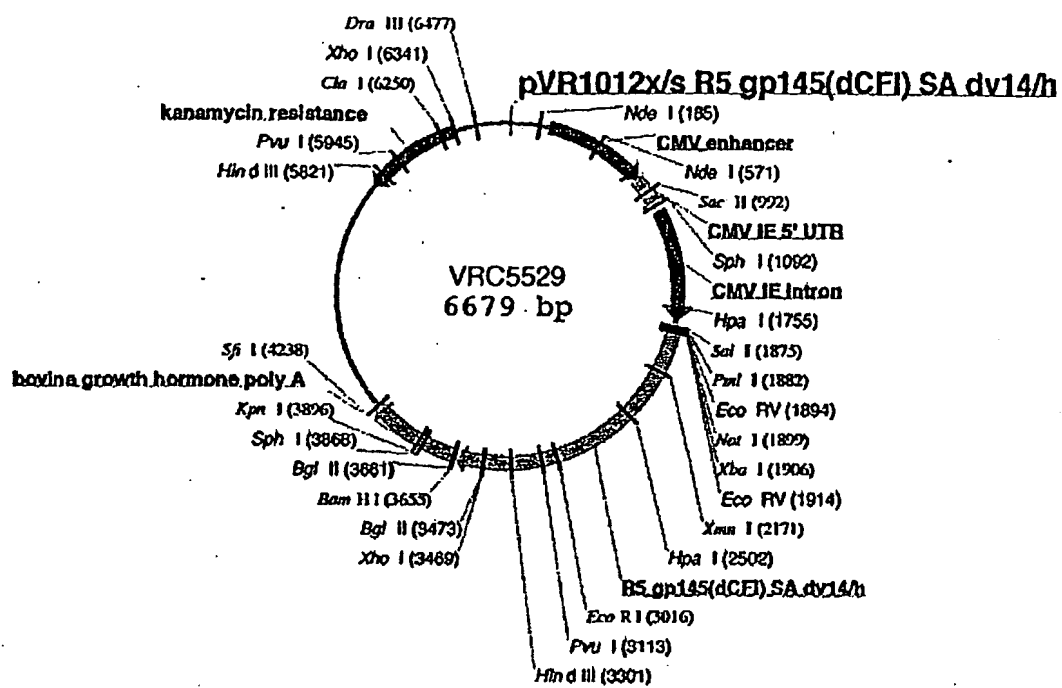


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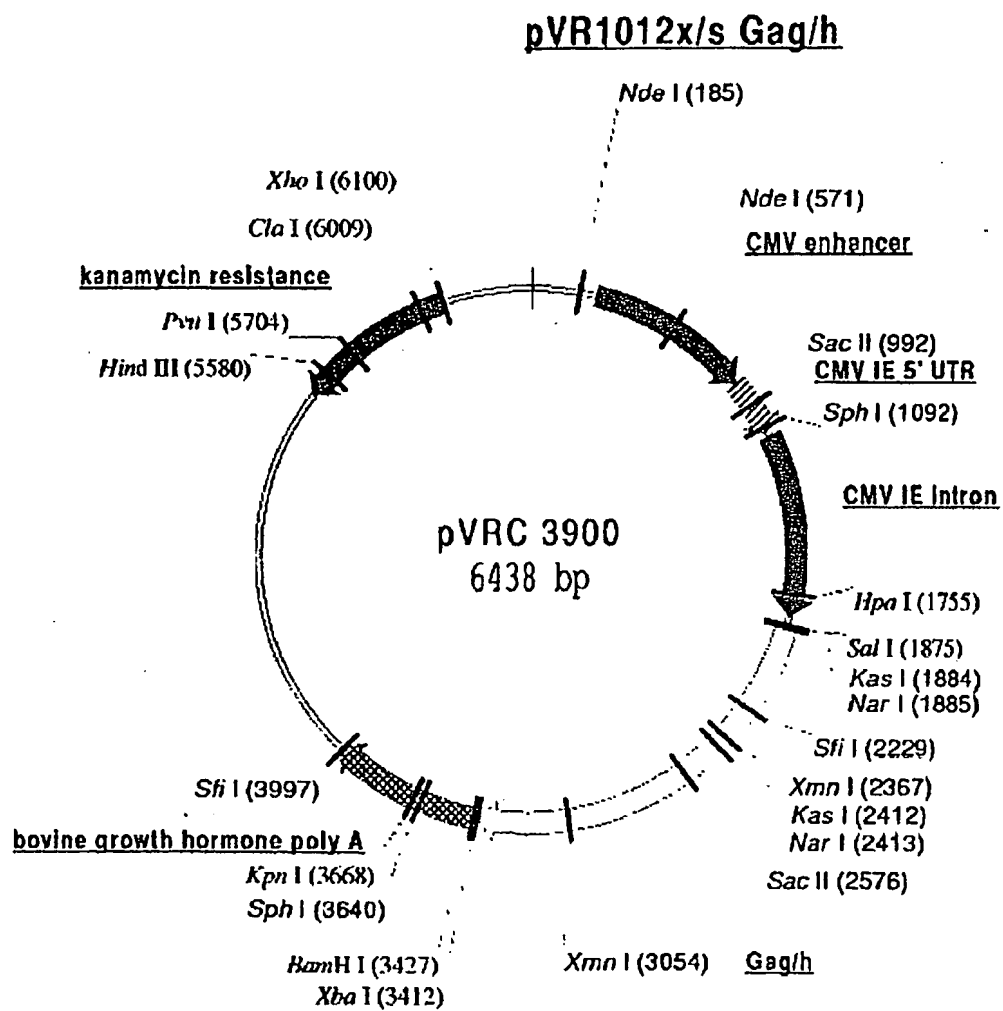


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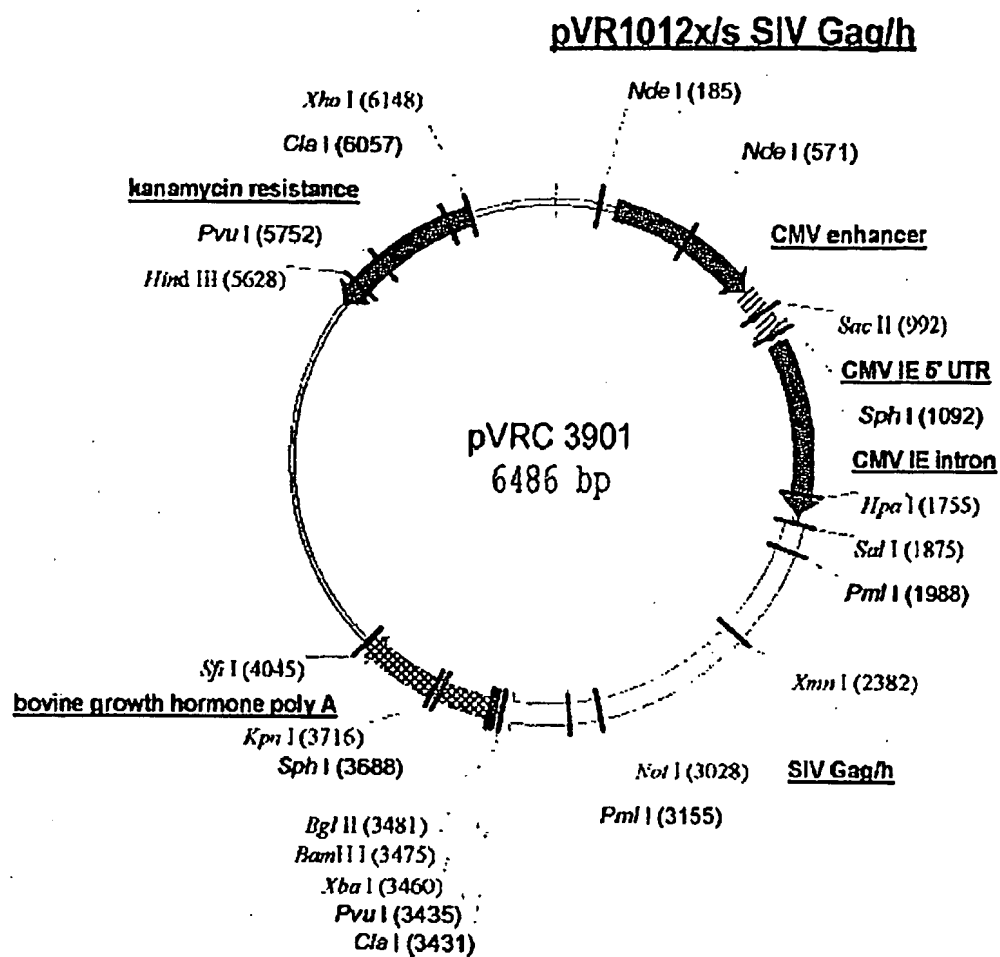


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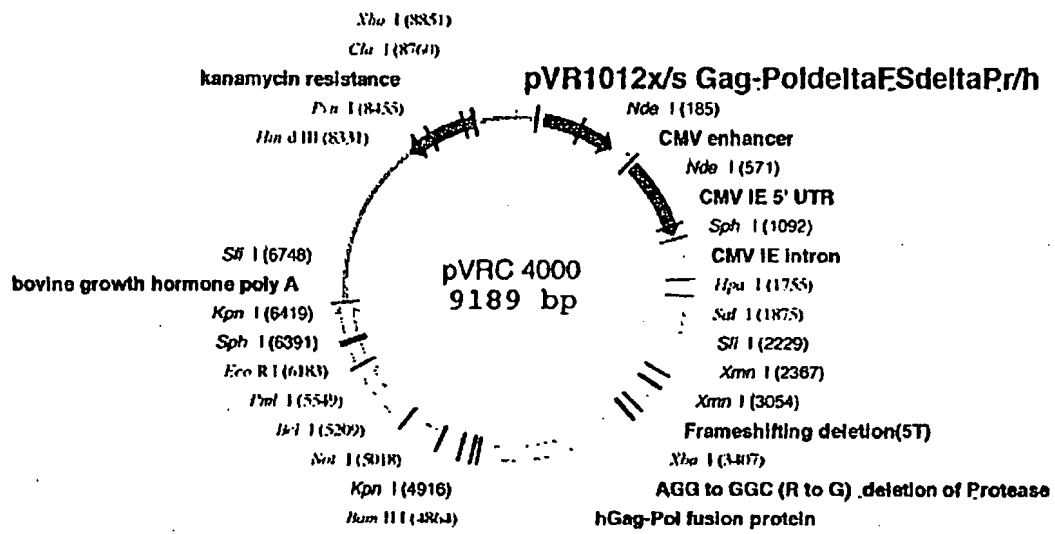


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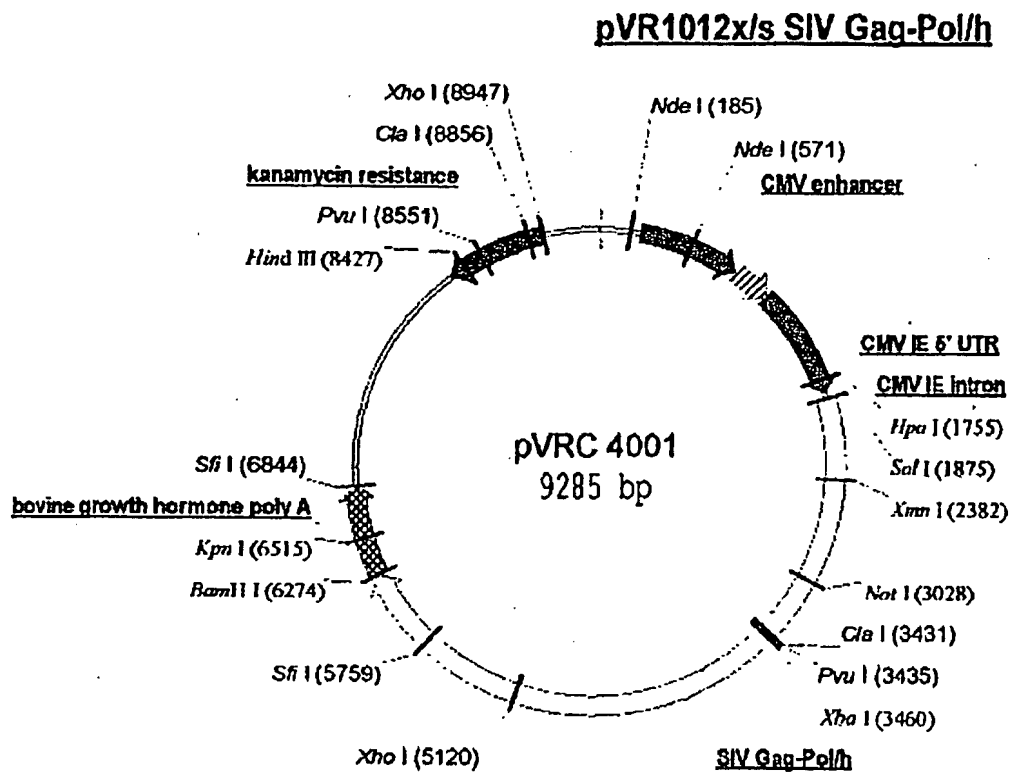


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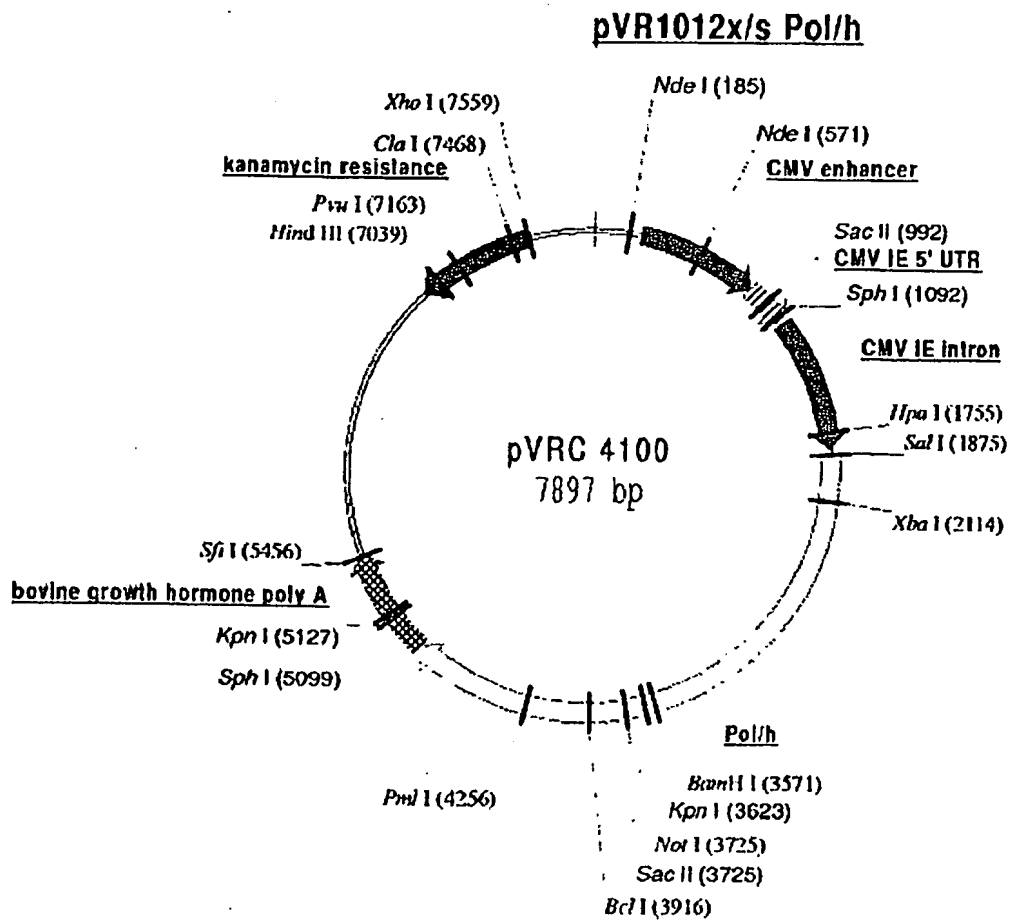


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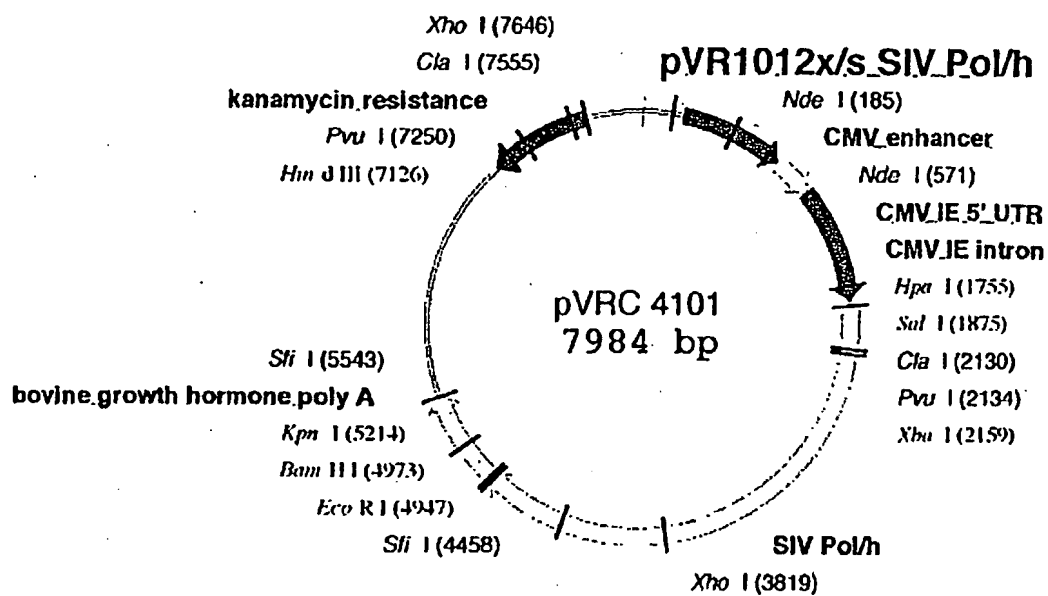


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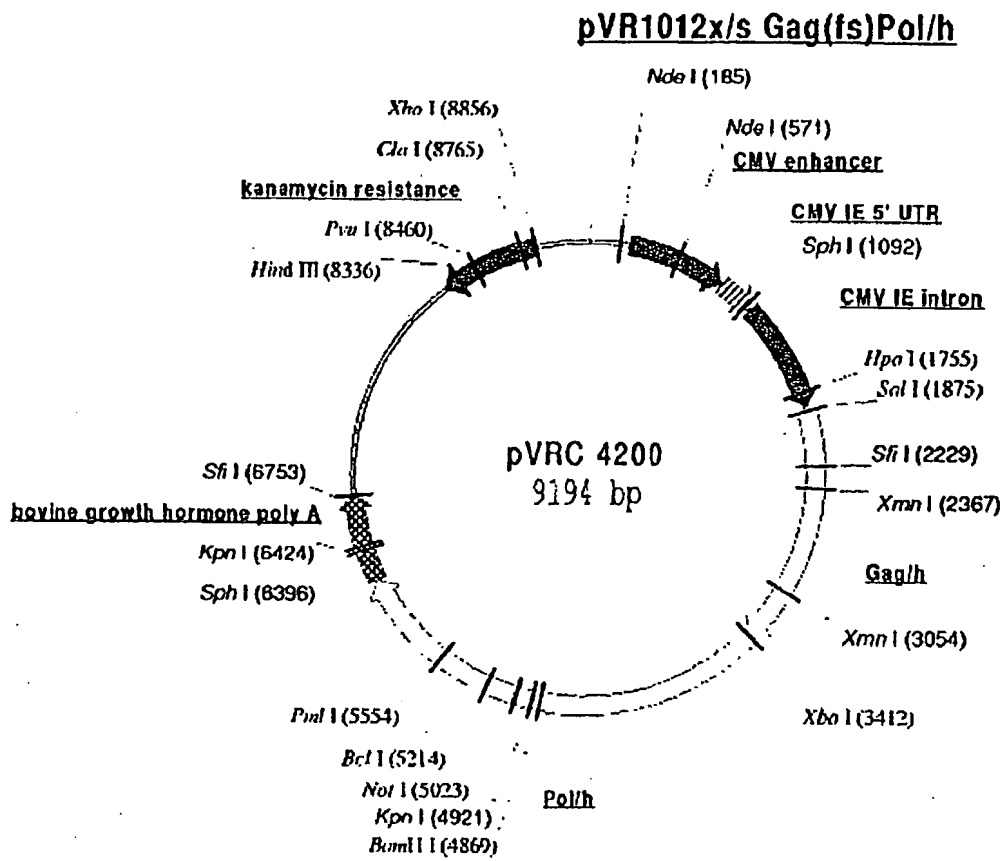


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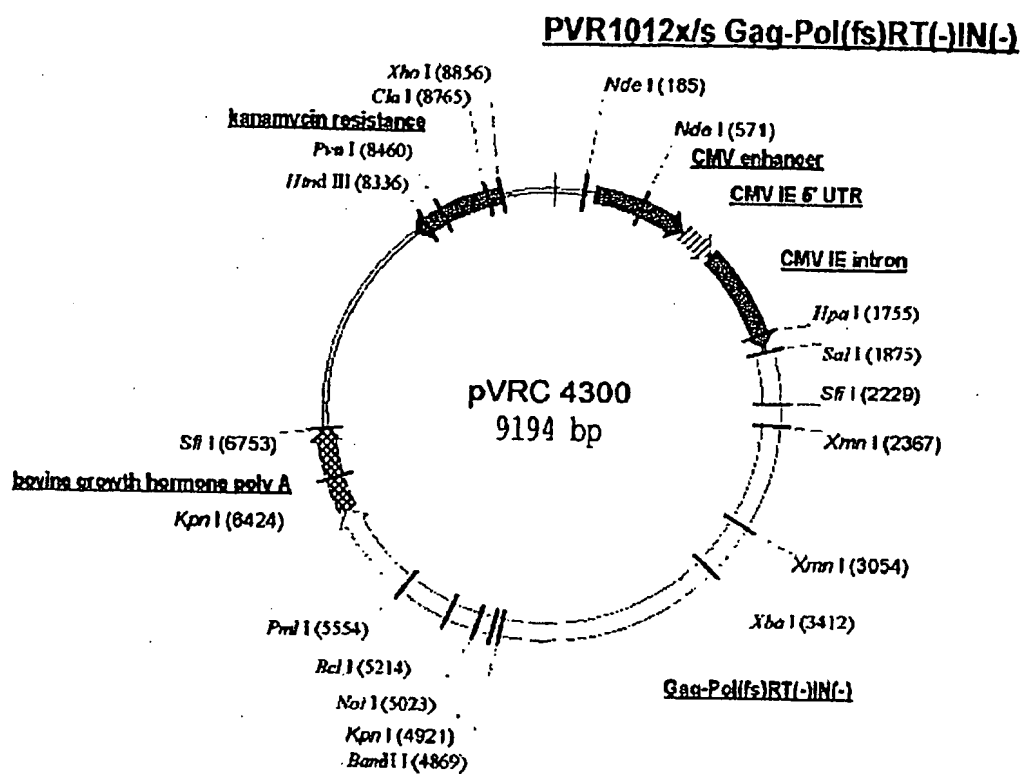


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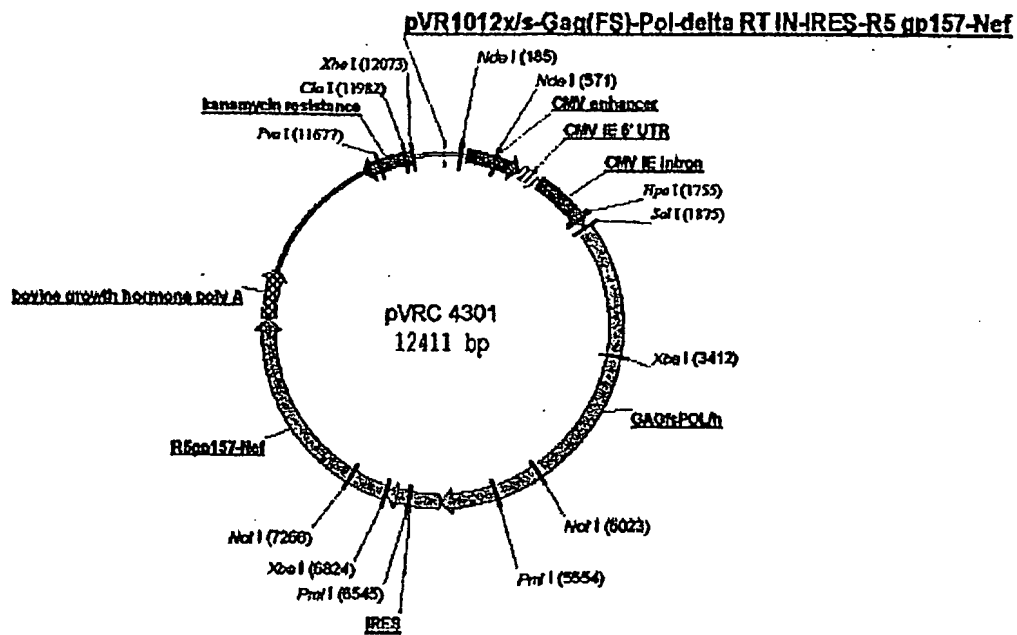


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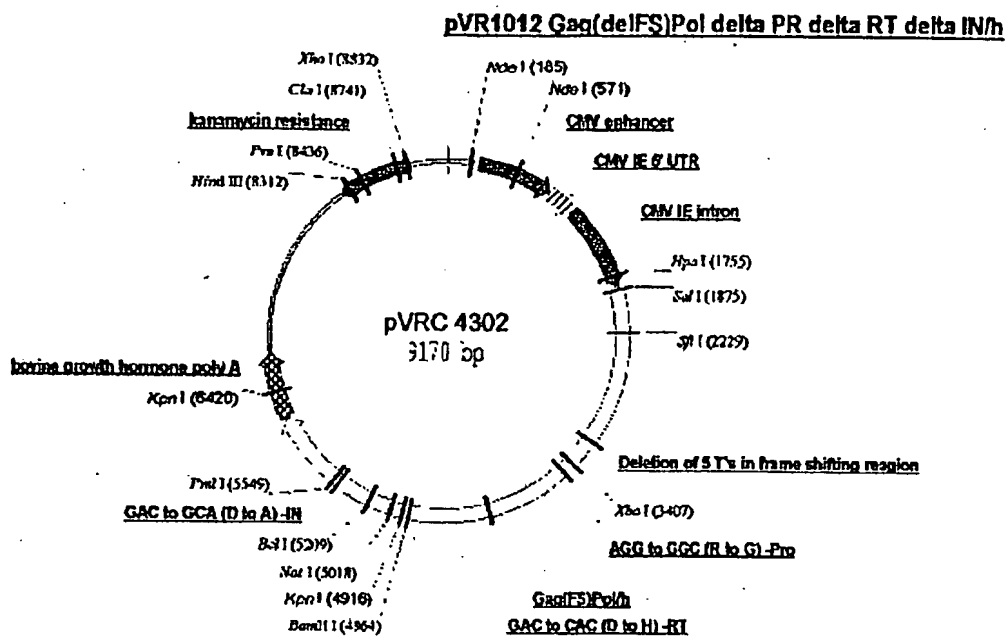


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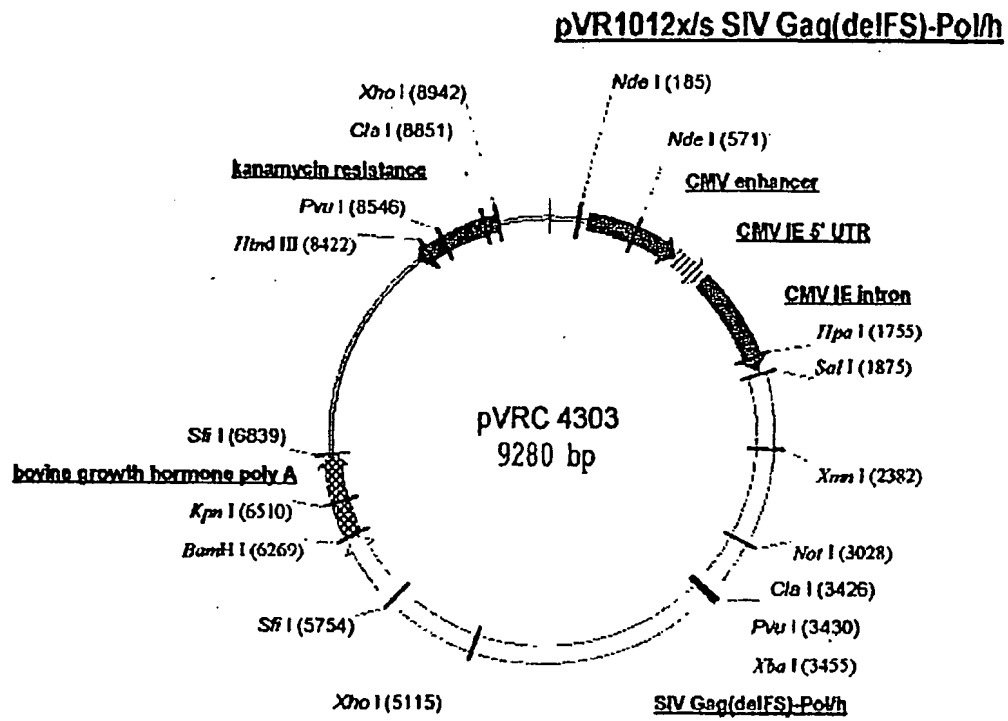


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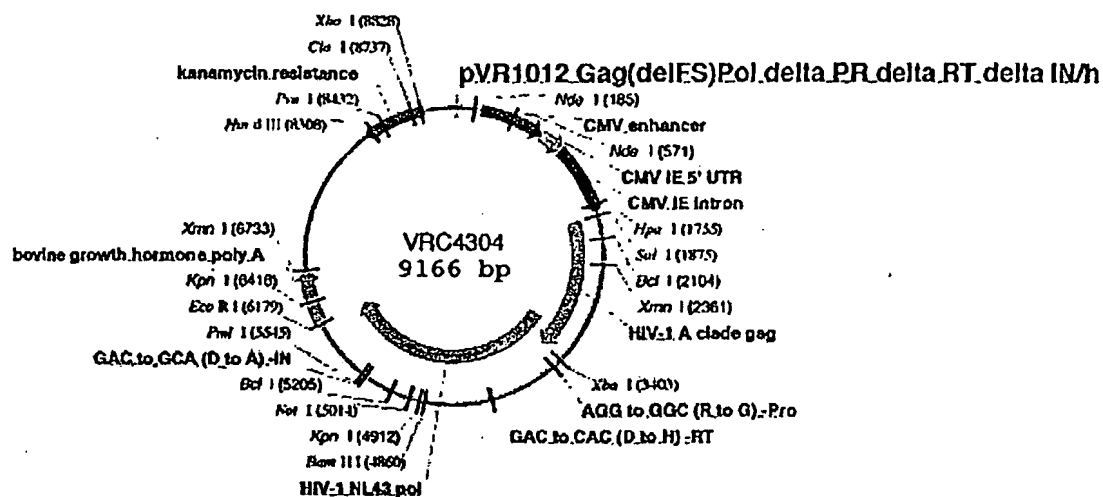


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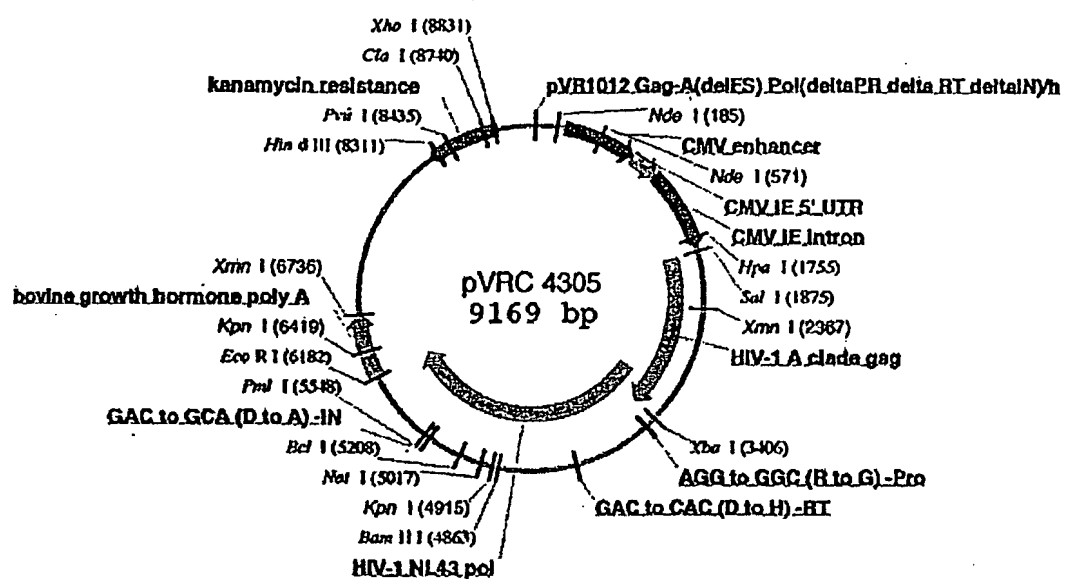


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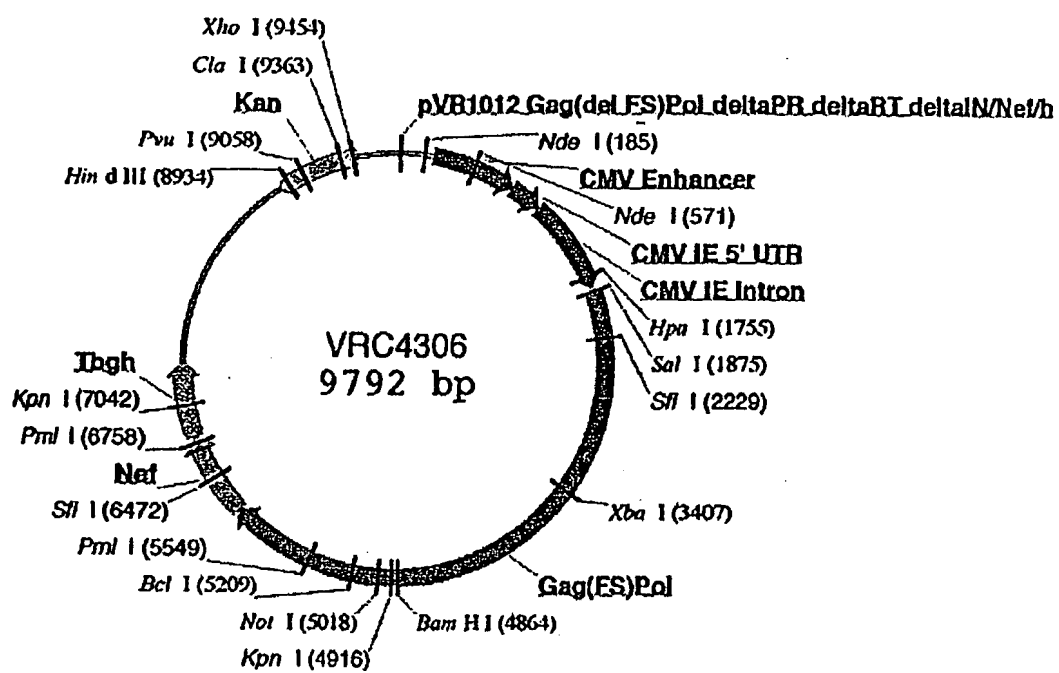


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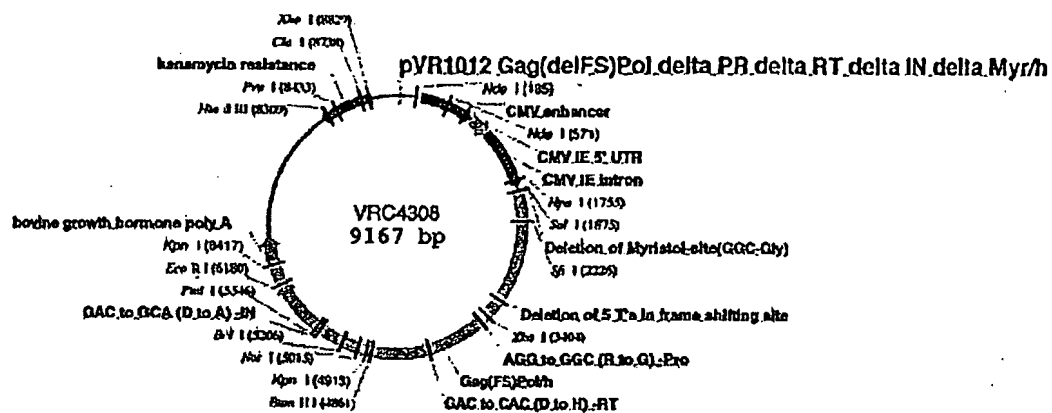


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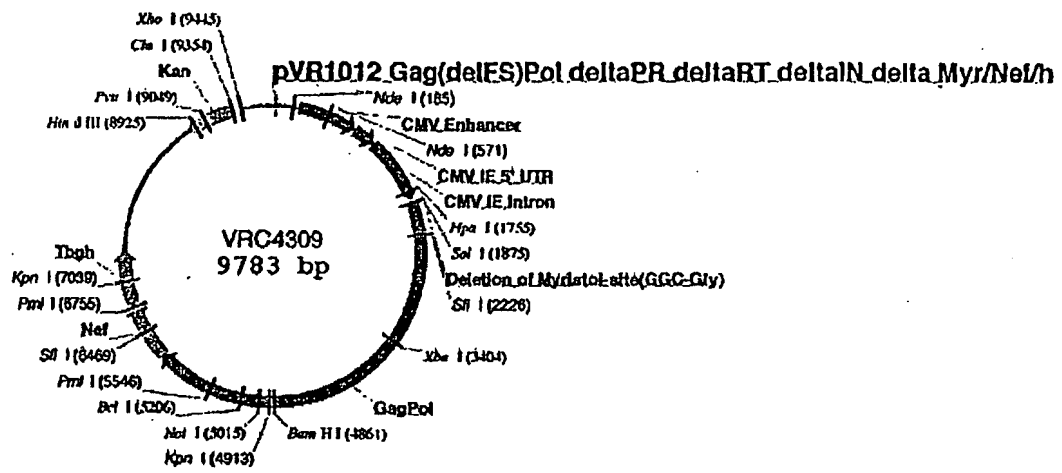


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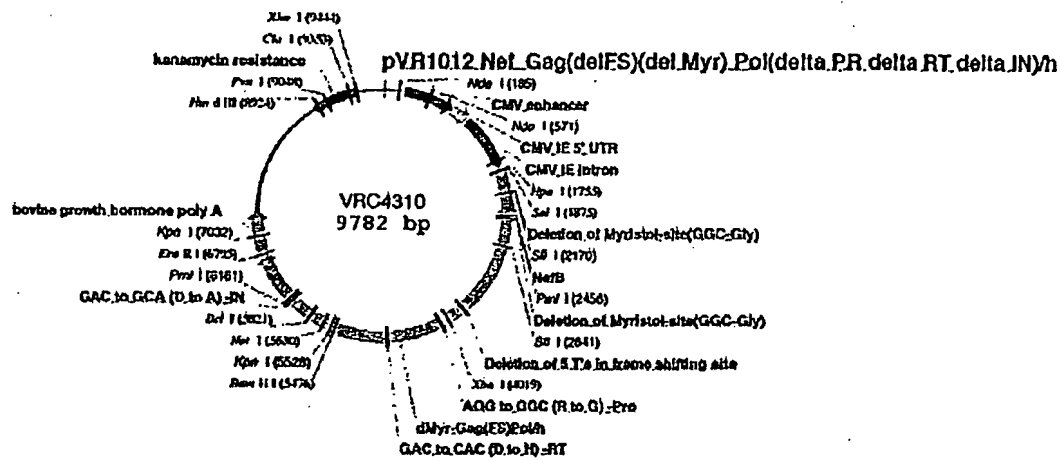


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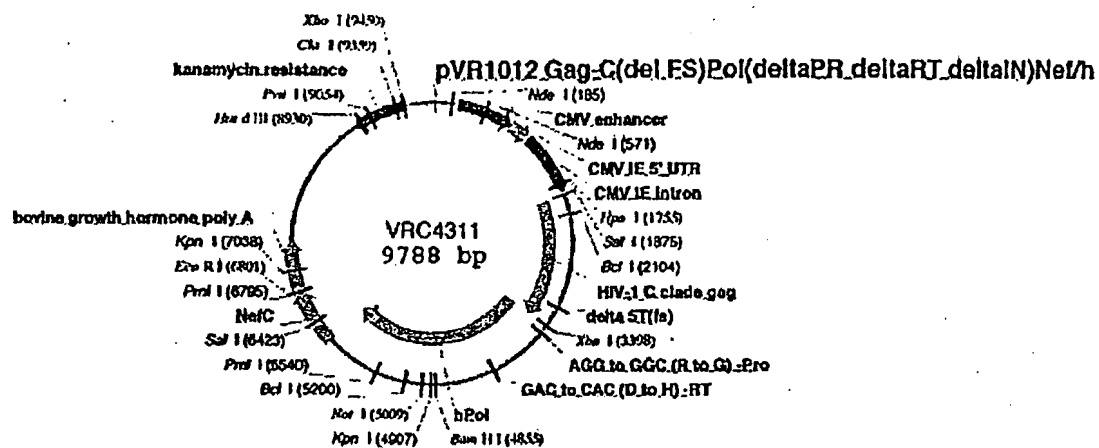


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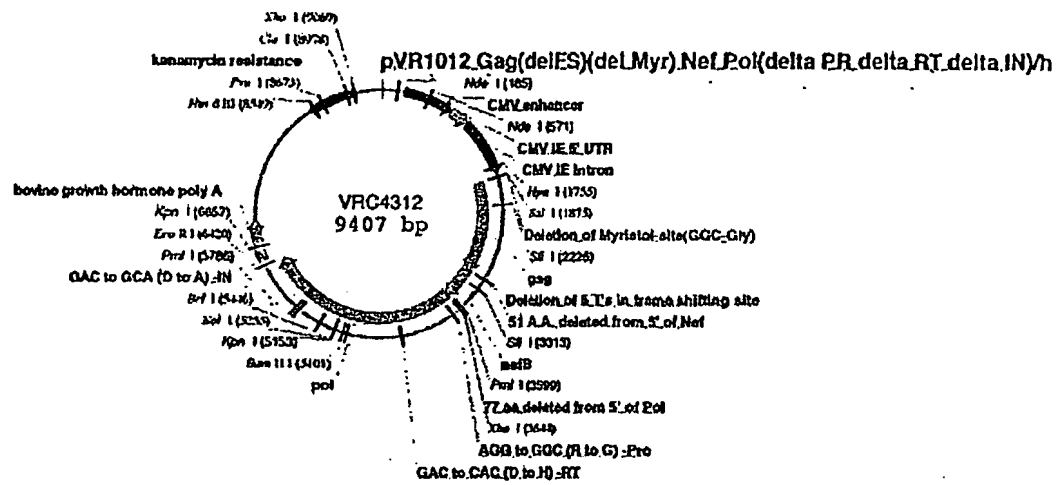


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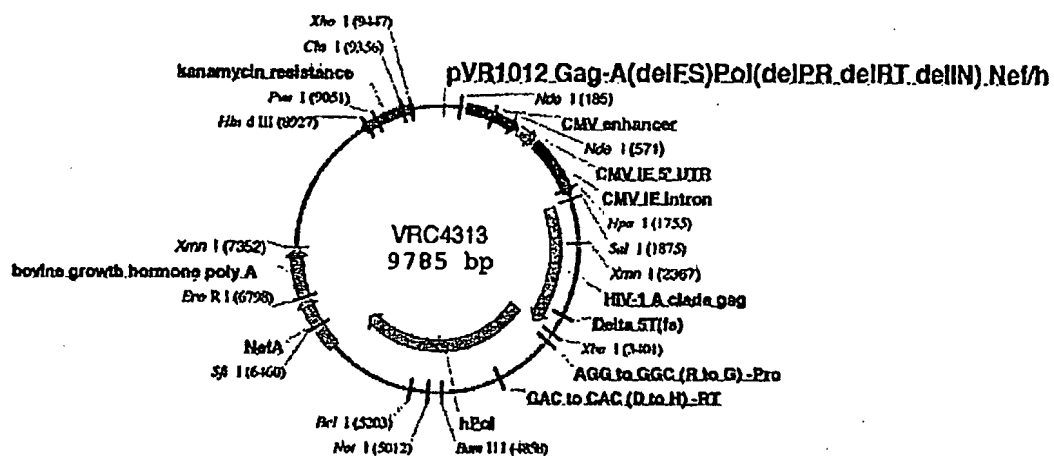


Figure 176

SEQUENCE LISTING

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CHAKRABARTI, Bimal K.
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<211> 5549

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<213> Artificial Sequence

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<223> plasmid pVR1012x/s containing HIV genes

<400> 82

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